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Study of the lipid requirements of the young dairy calf

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STUDY OF THE LIPID REQUIREMENTS OF THE

YOUNG DAIRY CALF

by

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I. INTRODUCTION

The relationship of lipids to the nutrition of the dairy calf has been the subject of experimental work for over half a century. For the most part these studies have been designed to find a cheap substitute for milk fat. Research has shown that calves cannot tolerate highly unsaturated crude vegetable oils, such as, soybean, cottonseed and corn oils. When such oils are included in reconstituted milks and fed to young calves, poor growth, severe diarrhea, and a high incidence of morbidity and mortality result. Hydrogenated vegetable oils, lard and tallow have been found to be more satisfactory. Similarly, skim milk and other low fat whole milk replacements are acceptible to the calf, but due to the lower energy content resulting from the low fat level, growth rate usually is less than for calves fed whole milk. In most studies of whole milk replacements, critical evaluation of dietary fat requirements has been impossible due to inclusion of supplements such as hay and grain and/or to inadequate length of experimental period. There have been no studies to determine whether calves require dietary lipids and to discover the manifestations, if any, of fat deficiencies.

The relatively recent development of equipment and methods to study the polyunsaturated acids has made the present investigation feasible. Factors which have served to focus attention on this problem are (1) certain feeds are becoming increasingly lower in fat content due to the new solvent fat-extraction procedures, (2) equipment for extracting butter fat from milk has been greatly improved, (3) most whole milk replacement formulas for calves are extremely low in fat and (4) more fundamental information on fat metabolism, and especially the role of the polyunsaturated fatty acids and phosphatides, in the calf is needed.

The objectives of this study were to determine whether the unsaturated fatty acids, linoleic, linolenic and arachidonic, are essential in the diet of the young calf and to study the role of phospholipids and neutral fats in the nutrition of the dairy calf.

II. REVIEW OF LITERATURE

A. Filled Milks

Various aspects of fat utilization by calves have been studied for many years. The low price of vegetable oils has encouraged their substitution in calf rations for the more expensive butterfat. A report of such substitutions was made as early as 1894. At that time, Lindsey (88) obtained fairly good growth in the calf when tallow and oleomargarine were combined with skim milk and brown sugar.

Crude soybean oil has been found to be markedly inferior to milk fat in oil-filled milks for young dairy calves (14, 59, 60, 78, 97, 114). Gullickson _et al_. (60) found that calves could not tolerate crude soybean oil at the 3.5 per cent level in skim milk. Murley _et al_. (97) observed that calves receiving crude soybean oil made inferior daily gains. Frequent and severe diarrhea and lethargy were noted. Barker _et al_. (10) noted a high incidence of scouring, retardation of growth, rough haircoat, excessive morbidity and high rate of mortality among calves fed crude soybean oil. Jacobson _et al_. (78) found calves were able to tolerate crude soybean oil at a two per cent level in the skim milk but growth was poor and there was a high incidence of diarrhea.

Corn and cottonseed oils apparently are similar to crude soybean oil in their effect on calves (59, 60, 61). Gullickson _et al_. (59, 60) concluded that neither corn nor cottonseed oil produced satisfactory results when used as a source of fat in the ration of young dairy calves. The growth rate was slow and incidence of diarrhea and mortality was high. Haecker (61) found rations containing two and three per cent corn oil to be too laxative.

Graham _et al_. (55) found that herring oil which contains a high percentage of unsaturated fatty acids was highly toxic when fed to goats, whereas this oil upon hydrogenation did not produce adverse reactions. This finding has been substantiated in studies of other highly unsaturated fats. Jarvis and Waugh (80) observed that growth and health of calves fed a purified diet containing hydrogenated cottonseed oil were better than those of calves fed a similar diet containing refined unhydrogenated oil. Jacobson _et al_. (78) concluded from calf feeding experiments that hydrogenated soybean-oil-filled milk yielded results comparable to whole milk or butter-oil-filled milk. Other workers (10, 97) have observed that the nutritive value of soybean oil is enhanced by hydrogenation and that calves tolerate saturated fats better than unsaturated fats.

Gullickson _et al_. (59, 60) found that calves receiving lard or tallow in skim milk at a 3.5 per cent level very closely approached those fed butter oil in growth and general appearance. Hayden (69) indicated that 10-day-old calves can be raised to vealing age more economically on skim milk plus a commercial milk fat substitute, largely beef fat, than by feeding whole milk. Wiese _et al_. (132) reported that animals fed lard at the rate of 3.5 per cent in a synthetic milk were healthy and normal in appearance.

Bate _et al_. (14) reported that feeding either soybean oil or butter oil separate from the remainder of the ration (skim milk) caused development of dermatitis and loss of hair. The hair on the inside of the rear legs began to fall out and the skin began to crack on the calves at two weeks. This alopecia and dermatitis could be prevented either by homogenizing the oils into the skim milk or by allowing the calves to eat hay and grain. Gullickson _et al_. (59), on the other hand, observed alopecia when calves were fed either corn or cottonseed oil despite homogenization of the fat.

B. Semi-synthetic Milks

Development of semi-synthetic milks is of recent origin. The initial studies of developing synthetic milks were begun by Clark (30) in 1927. Before this time, synthetic milk had been considered more as a curiosity than as a basis for research. Analyses of milk were not sufficiently complete and there was little information regarding the nature of even simple systems in which phosphate, calcium and citrate take part simultaneously in acid-base equilibria.

Clark (30, p. 197) after studying the limited knowledge of the true chemical composition of milk assumed the composition to be as given in Table 1.

Table 1

Composition of Milk

Components	Grams per Liter
K_2O	1.80
Na_2O	0.72
CaO	1.78
MgO	0.30
P_2O_5	1.50
Citric acid	2.00
Cl	1.00
SO_3	0.11
Casein	28.00
Albumin	7.20
Other proteins	0.20
Lactose	50.00
Fat	0.00

Clark (30, p. 198) used this information to prepare a synthetic milk. The following method was the most successful.

	Moles	Grams	
Solution I			
	.0074 MgO	.298	Dissolve with warming and dilute to 100 cc.
	.0211 KH_2PO_4	.873	
	.0104 Citric acid · H_2O	2.185	
Solution II			
	.0014 $CaCO_3$	0.140	Dissolve cold. Dilute to 50 cc. and use while fresh.
	.0014 H_2SO_4	28 cc. 0.1 N	
Solution III			
	.0141 $CaCl_2$	1.565	Dilute to 50 cc.
Solution IV			
	.0171 KOH	17.1 cc. 0.1 N	Dilute to 50 cc.
	.0232 NaOH	23.2 cc. 0.1 N	
Solution V			
	7 grams casein and 10 grams lactose in 130 cc. 0.20 N $Ca(OH)_2$		For 200 cc. "synthetic milk" in which casein represents total protein.

To prepare a lime-water as strong as 0.20 N thoroughly saturate the solution while it is cooled with surrounding ice. Add this slowly to the casein, while the casein is being ground in a deep glass mortar. Finally stir with a motor-driven stirrer.

Add 10 cc. of Solution IV. When the solution is free from suspended material add 10 grams lactose. As soon as this is dissolved add at once 10 cc. of Solution III and 10 cc. of Solution II. Now set the motor at high speed and to the rapidly stirring solution add very slowly from the fine tip of a pipette 20 cc. of Solution I. When this addition is complete dilute the "milk" to 200 cc. "Milks" prepared in this manner have the appearance of a very "blue" skim milk.

This milk was stable when centrifuged at 2500 RPM for 30 minutes and was readily coagulated by rennet. The writer was unable to find any evidence that this milk has been fed to any class of animals.

Early studies revealed that calves could not long survive if whole milk was the sole source of nutrients. Animals maintained on whole milk diets for prolonged periods were not healthy in appearance and exhibited digestive disturbances, anemia, tetany, lack of appetite and poor growth. Duncan et al. (37) discovered that magnesium prevented the development of tetany. Wise et al. (135, 136) demonstrated that if the whole milk ration was further supplemented by iron, copper and vitamins A and D many of the abnormalities could be overcome. Such supplementation had no remedial effect upon appetite.

The earliest attempt to raise colostrum-fed calves on semi-synthetic milk was made by Johnson et al. (82) at Cornell University. Calves two to ten days old were fed a purified ration consisting of an artificial mixture of crude casein, lactalbumin, sugar, butter or lard, minerals and water. A dry mixture of starch, sugar, cottonseed oil, cellophane and minerals was kept before the calves after the first few days and they were transferred completely to the dry feed after about three months. Some of the calves were raised to five and six months of age but the mortality rate was high and those that did survive made subnormal gains. Some calves died in tetany. In subsequent experiments the tetany was prevented by feeding $MgCO_3$ but a number of the animals which developed paralysis of the hind legs were not benefited by magnesium feeding. The growth rate of these calves was below normal when compared to the Ragsdale Standards (106). Reduced feed intake associated with periodic digestive upsets seemed to be largely responsible for the slow growth.

Wiese _et al_. (132) reported the development of a semi-synthetic milk which promoted good growth in colostrum-fed calves. The "synthetic" milk was prepared by dissolving casein in a water solution of $NaHCO_3$. After the addition of lard, cerelose and mineral salts, water was added to give a solids content of 13 per cent. Thirteen vitamins were used to supplement the basal ration. It was reported that animals receiving this milk grew well, seldom scoured and appeared thrifty. A few animals were changed to a dry diet of casein, lard, mineral salts and cerelose after they had received the liquid milk for six weeks. The animals would not eat the dry ration except when fed as a slurry. On this ration diarrhea and loss of weight were observed but the substitution of starch for some of the cerelose improved the ration.

Kastelic et al. (84) experimented with two semi-synthetic milks. Milk I (Table 2) (Ca:Na ratio 0.7:0.9) was prepared as described by Wiese et al. (132) with some modification. Kastelic et al. (84) used a water emulsion of soy lecithin and cottonseed oil prepared in a Waring blendor, adding this to the milk after the casein, egg albumin, sugar and salts were brought into solution.

Table 2

Composition of Synthetic Milk I

Components	Grams per Kg. Final Liquid Milk
Crude casein	35.0
Cerelose	50.0
Cottonseed oil	10.0
Egg albumin	8.0
Salts	4.0
Soy lecithin	2.0

All known vitamins except B_{12} were added in generous amounts. The synthetic milk was not coagulated by rennet. Calves receiving this milk scoured almost continuously and all attempts to control the diarrhea were unsuccessful.

The Milk II (Table 3) was similar to that prepared by Clark (30) except that 0.1 N HCl was used to adjust the

Table 3

Composition of Synthetic Milk II

Components	Grams per Kg. Final Liquid Milk
Crude casein	35.0
Cerelose	50.0
Cottonseed or butter oil	25.0 or 35.0
Salts	2.0
Citrus pectin	2.0
Soy lecithin	2.0

final pH of the milk to 6.6. A water emulsion of soy lecithin and trace minerals and vitamins were added as described in preparation of Milk I. Cottonseed oil or butter oil, when fed, was emulsified in an aqueous suspension of soy lecithin before addition to the milk. This milk was coagulated by rennet in about three to five minutes.

Calves started on this ration from birth made larger daily weight gains than would be expected according to Ragsdale Standards (106). With some exceptions, calves were fed this milk from 0 to 20 days and they received twice daily all the milk they would drink willingly. The calves were housed in an air conditioned room where no calves had previously been kept. The pens were cleaned thoroughly each day and rebedded with sawdust. The calves received subcutaneous injections of streptomycin during the first few days of life. Very little diarrhea was observed.

Draper et al. (36) maintained calves on an alpha protein "synthetic milk" diet supplemented with methionine, lard, cerelose, minerals and vitamins excepting vitamin B_{12}. These calves grew poorly, had poor appetites and in some cases exhibited a lack of coordination. Liver extract or crystalline B_{12} therapy in some instances restored growth but in other cases no response was obtained.

Jarvis and Waugh (80) found that young calves did not grow well when fed purified rations. The calves appeared healthy when a purified diet containing 3.5 per cent hydrogenated cottonseed oil was fed but they developed fatty livers. It was concluded that the purified diets were conducive to fatty livers since animals had normal livers when they were fed hydrogenated cottonseed oil reconstituted with non-fat milk solids.

Flipse et al. (50) found corn sugar to be superior to dextrin or corn starch when fed to calves from 5 to 36 days of age. In a subsequent investigation Flipse et al. (51) divided 18 calves into three groups and fed synthetic milks which varied only in the source of carbohydrate. The synthetic milks were prepared by a modification of the procedure of Wiese et al. (132). The calves were allowed to remain with their dams for twelve hours following birth and then were placed in individual pens. Feed was given twice daily via nipple pail at a rate calculated to meet the recommended nutrient allowances suggested by the National Research Council (89). No hay or grain was fed.

The synthetic milk contained 25 per cent casein, 10 per cent lard, 5 per cent salts and 60 per cent carbohydrate. The average gain of animals which received carbohydrate in the form of glucose during the 31-day experimental period was 9.55 pounds per calf. Animals which received glucose and corn syrup, gained an average of 8.66 pounds and calves fed glucose and lactose, made average gains of 18.66 pounds during the same experimental period. The efficiency of feed utilization, expressed as the average gain per pound of dry matter consumed, was 0.234, 0.142 and 0.384, respectively. In subsequent work (52) conducted over similar experimental periods calves receiving lactose plus corn syrup gained an average of 28.44 pounds, those receiving lactose plus starch gained an average of 24.67 pounds, while calves fed starch averaged only 14.00 pounds gain. The efficiency of feed utilization, expressed as pounds of gain per pound of dry matter consumed, was 0.487, 0.412 and 0.204 for the three groups, respectively. The quantity of lactose required by the calf is apparently small because Flipse _et al_. (52) found lactose fed at the five per cent level produced almost as satisfactory growth response as did a 30 per cent lactose diet. The greatest efficiency of feed utilization was obtained at the ten per cent level of lactose but the growth rate difference was small.

C. Phospholipids

The presence of phospholipids in animal tissues has been known for over a century but their function in animal nutrition is not well known.

Lecithin is quantitatively the most important phospholipid in the plasma and serum of blood according to Wittcoff (137). Whole blood contains between 0.2 and 0.3 per cent phospholipid but wide variations have been observed in individual animals and in various species. The corpuscles contain small amounts of lecithin and relatively large amounts of cephalin and sphingomyelin.

Deuel (33) found that lecithin absorbs iodine, indicating lecithin contains at least one unsaturated fatty acid. He established that saturated acids, palmitic and/or stearic, were present in the lecithin molecule.

Recent reports indicate that the addition of lecithin results in a significant increase in the efficiency of vitamin A utilization and storage (12, 42, 43, 44, 77, 124, 125).

Crude soybean lecithin seems particularly active in this regard. Guerrant and Thompson (58) found that the biologically active material was associated with the non-saponifiable fraction of crude soybean lecithin. Chromatographic fractionation of the non-saponifiable residues and spectrophotometric examination of the eluates revealed the presence of carotenoids. Biological testing of the purified fractions revealed pro-vitamin A activity. Such evidence suggests that the "vitamin A sparing effect" of commercial soybean lecithins is attributable in part to the presence of carotenoids.

Experimental and clinical studies of fat absorption in humans by Jones et al. (83) demonstrated that the addition of an emulsifying agent to the diet improved fat utilization. These authors postulated that lowering of surface tension and improved emulsification of the fat were involved. Kastelic et al. (84) demonstrated that it was necessary to thoroughly homogenize cottonseed oil in the presence of soy lecithin to avoid digestive disturbances in the young calf, but that butter oil could be partially emulsified and successfully fed in the absence of soy lecithin. Tidwell (128) fed a fat containing 20 per cent lecithin to rats and found an increase in the chylomicrons in the blood. Adlersberg and Sabatka (2) reported that lecithin increased the blood fat levels in normal human subjects. Shantz _et al_. (119) observed that rats had better hair coats and appeared healthier when egg lecithin was fed with corn oil and coconut oil, suggesting that lecithin enhances fat utilization. Augar _et al_. (7) fed purified diets and observed that the addition of lecithin to the cottonseed oil or to the hydrogenated cottonseed oil markedly lowered the incidence of diarrhea and greatly improved the digestibility of these oils by the rat. The beneficial effect of lecithin on fat digestion was explained by suggesting that lecithin increased the rate and degree of fat emulsification.

Huff _et al_. (75) found glycerol-monostearate increased the absorption of lipids by young dairy calves as determined by "Allen fat"[1] levels in blood plasma. Calves receiving glycerol-monostearate made body weight gains which were not significantly superior to those made by the controls.

Shoskes _et al_. (122) could not verify these observations concerning the value of emulsifying agents in enhancing fat absorption in the rat. They reported that the absorption of corn oil was unaffected by the presence of purified soybean

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[1]Determination of blood fat using the Allen (3) method.

phospholipid or "tween 80" greatly in excess of that needed to make excellent emulsions of the fat by mechanical means.

Kellner et al. (85) reported that intravenous injection of a detergent (triton A-20) caused a five fold increase in blood phospholipids in the rabbit. They did not offer an explanation for the possible mode of action. These results are not in harmony with the observations of Chung and Shaw (29) which indicated plasma phospholipids were not increased when a surface active agent was intravenously injected into a cow and a goat. The concentrations of the other lipids were greatly increased.

Bernhart et al. (15) reported that the addition of 12.5 per cent lecithin to a skim milk and horse meat ration resulted in a reduction in clostridea in feces of rats after one week of feeding. Other studies showed that the removal of the lecithin from the diet caused fecal counts to rise to control values.

D. Blood Lipid Values

Abderhalden (1) in 1911 presented one of the earliest analyses for blood lipids on record (Table 4).

Mayer and Shaeffer (93) reported values for various lipids occurring in the blood serum of the cow as determined

Table 4

Serum Lipids in Bovine Blood

	Cholesterol	Lecithin	Fatty Acids	Fat
	(parts per thousand parts by weight)			
Cow	1.238	1.675	--	0.926
Bull	1.901	1.869	0.743	3.542

Table 5

Serum Lipids in Bovine Blood

	Total Fatty Acids	Phospholipid[1]	Cholesterol
	(mg./100 ml.)		
Cow	171	93	89

[1]Phospholipid = P x 25.

by the Kumagawa-Suto method for total fatty acids, the Windaus method for cholesterol and calculated phospholipid from ether soluble phosphorus. These are shown in Table 5.

The values obtained by Bloor (17) for total fatty acids and for unsaponifiable materials are summarized in Table 6.

Allen (3) developed a procedure for the quantitative estimation of blood fat and studied a number of blood samples

Table 6

Serum Lipids in Bovine Blood

	No. of Samples	Total Fatty Acids		Unsaponifiable	
		Average	Range	Average	Range
		(mg./100 ml.)			
Beef	16	154	(102-206)	104	(67-184)

taken from calves before their first nursing. His method measures essentially all the lipids except phospholipids and free fatty acids. This method of analysis failed to detect blood fat in calves which had not nursed. This author also reported that the blood fat values of 35 calves of the Holstein, Jersey, Guernsey and Ayrshire breeds averaged 123 mg. per 100 ml. of blood plasma at one month and 159 mg. at two months of age. It was established that the plasma fat increased rapidly during the first week after birth when the calves were consuming large quantities of whole milk. The fat content of the plasma increased until the calves were about six weeks old. At this age the ration was changed to skim milk, hay and grain and a gradual decline in blood fat was observed during the following six weeks. An increase in blood fat then occurred, gradually increasing until the calves were about one year old.

In 1939, Wise et al. (136) reported that the amount of fat consumed markedly influenced the fat content of plasma. The fat content was "practically nil during the early stage of life". Between the ages of 55 and 324 days the blood fat level gradually increased to approximately 200 mg. per cent providing the calf remained healthy: otherwise, the trend was up for a short time and then downward and variable. Digestive disturbances frequently caused marked reductions in plasma fat.

Gullickson et al. (60) observed higher blood fat levels in calves receiving corn oil or cottonseed oil than among those receiving butter fat. Calves receiving either corn oil or cottonseed oil had plasma fat levels ranging from 160 to 340 mg. per cent over a ten-week period. Animals receiving milk fat in their rations had plasma fat concentrations from 120 to 210 mg. per cent during the same period. These values were inversely related to the rate of body weight gains. The high blood fat level indicated that these oils were absorbed from the intestinal tract; nevertheless growth performance was poor.

Murley (97) also found that type of dietary fat markedly influenced the plasma fat levels of calves over the age period from 4 to 60 days of age (Table 7). The blood fat levels of calves receiving crude soybean oil were highest,

Table 7

Effect of Type of Oil in Filled-Milk Diets of Young Calves on Mean Concentrations of Fat in Blood Plasma[1]

Dietary Group[2]	Age in Days								
	4	11	18	25	32	59	46	53	60
	(mg./100 ml. plasma)								
Butter oil	87.4	47.4	114.0	147.0	132.2	120.0	138.4	135.0	144.6
Crude soybean oil	82.4	76.4	145.8	206.6	194.4	171.0	191.0	164.0	166.6
Refined soybean oil	112.6	82.6	169.0	233.2	229.0	222.2	195.0	186.2	185.4
Hydrogenated soybean oil	112.8	30.6	71.0	74.4	64.6	56.0	63.6	60.4	51.2

[1]Allen fat technique (3).

[2]Five calves in each group.

those of calves fed butter oil were intermediate and those of calves fed hydrogenated soybean oil were lowest. These data and those of Gullickson _et al_. (59) and Wise _et al_. (136) fail to reveal any consistent relationships between blood fat levels and growth rates.

Huff _et al_. (75) fed three groups of five calves each from the colostral stage to six weeks of age. The calves were fed synthetic rations (Table 8) which differed only in the agent used to emulsify the fat. Glycerol-mono-stearate was added as an emulsifying agent in diets B and C. Diet C was identical to diet B except that the fat in it was not homogenized. The fats in diets A and B were homogenized. The average blood plasma fat values, "Allen fat" procedure, for the three groups are indicated in Table 9.

At four and six weeks of age plasma fat values of calves receiving diet B were significantly higher than those of calves receiving diet A. The plasma fat levels of calves fed diet C were intermediate. The body weight gains of calves fed diet A were somewhat better than those of calves fed either diet B or C but the differences were not significant statistically. All calves on diet C, unhomogenized milk, lost hair around the anus, on the tail and on the inside of the thighs.

Chung and Shaw (29) found intravenous injections of a surface-active agent resulted in a marked increase in all

Table 8

Composition of the Diets Fed to Young Dairy Calves

Constituents	Diet A (homogenized)	Diet B (homogenized)	Diet C (unhomogenized)
		(per cent)	
Casein	4.0	4.0	4.0
Glucose	5.5	5.5	5.5
Salt mixture	1.25	1.25	1.25
Hydrogenated cottonseed oil	3.5	3.0	3.0
Glycerol-mono-stearate	--	.5	.5
Water	85.75	85.75	85.75
Vitamins	(added)	(added)	(added)

Table 9

Average Blood Plasma Fat Values of Calves
on Various Dietary Regimens

Dietary Group	Average Plasma Fat		
	2 weeks	4 weeks	6 weeks
	(mg./100 ml.)		
A. Homogenized	85.9	98.2	100.8
B. Homogenized and glycerol-monostearate	109.7	165.1	151.9
C. Glycerol-monostearate	101.7	119.0	132.0

plasma lipids except phospholipids in a goat and a cow. The total plasma lipids of the cow increased to five times normal, neutral fat increased to 17 times normal and both free and ester cholesterol increased to four times normal. Total plasma lipids reached 2,049.2 mg. per cent and remained above 1,000 mg. per cent for nine days without producing an increase in milk fat production.

Jacobson et al. (79) fed a variety of dietary fats to calves which previously had received whole milk (three per cent fat) for two weeks. One group continued to receive whole milk. The other four groups received homogenized reconstituted milks containing three per cent butter oil, lard, crude soybean oil and hydrogenated soybean oil, respectively. The results are shown in Table 10.

During the preliminary two week period when the calves were receiving three per cent whole milk the average blood lipid values in mg. per 100 ml. of plasma for the 15 calves were; total lipid, 221; phospholipid 39.6; and "Allen fat", 173.

Zaletel et al. (140) studied the blood plasma lipids of eleven calves during the period from birth to four days of age and reported the values shown in Table 11.

Increases in the values for total lipids, ester fatty acids, neutral fat, ester cholesterol and phospholipids were

Table 10

Effect of Type of Dietary Lipid upon the Concentrations of Lipids in Blood Plasma[1]

Dietary Lipid	Total Lipid	Phospholipid	"Allen Fat"
	(mg./100 ml.)		
Hydrogenated soybean oil	135	14	102
Crude soybean oil	267	38	232
Lard	196	38	163
Milk fat (butter oil)	199	30	147
Milk fat (whole milk)	285	70	233

[1]Average of values at 3 and at 4 weeks after change to experimental diet.

Table 11

Blood Plasma Lipids in Young Dairy Calves

Plasma Lipid	Age in Days		
	0 (birth)	2	4
	(mg./100 ml. plasma)[1]		
Total lipid	59 ± 6	132 ± 12	151 ± 13
Ester fatty acids	34 ± 4	75 ± 9	82 ± 9
Neutral fats	23 ± 3	38 ± 6	37 ± 7
Ester cholesterol	11 ± 2	32 ± 2	38 ± 6
Free cholesterol	5 ± 1	7 ± 1	10 ± 1
Phospholipid	7 ± 3	29 ± 5	31 ± 5
Free fatty acids	8 ± 3	8 ± 2	10 ± 2
"Allen fat"	25 ± 3	96 ± 11	111 ± 11

[1]Mean values ± standard error.

Table 12

Total and Polyunsaturated Fatty Acids in Calf Blood Plasma

Age in Days	Total Fatty Acids	Linoleic	Linolenic	Tetraenoic
	(mg./100 ml. plasma)			
0	27.8	2.8	0.6	3.2
1	58.1	12.3	1.5	5.9
2	86.0	20.2	1.8	7.2
3	103.8	26.9	3.6	7.1
4	98.4	28.2	2.7	7.3

observed but no marked changes were apparent in free cholesterol and free fatty acid concentrations.

Work on bovine blood plasma levels of the polyunsaturated fatty acids is limited. Allen _et al_. (5) in studies of polyunsaturated fatty acids in the blood plasma of five calves from birth to four days of age reported the values shown in Table 12. The data show that the levels of all polyunsaturated fatty acids in calf plasma were lower at birth than on subsequent days. O'Connell and Daubert (99) were unable to demonstrate the presence of linoleic acid in beef plasma lipids. Allen and Zaletel (4) using alkali conjugation techniques analyzed calf blood plasma for linoleic, linolenic and tetraenoic fatty acids and reported average values of 44.2, 4.8, 10.8 mg. per cent for linoleic,

Table 13

Effect of Dietary Lipids on the Polyunsaturated Fatty Acids in Blood Plasma of Young Dairy Calves

Dietary Lipid	Linoleic	Linolenic	Tetraenoic
	(mg./100 ml. plasma)		
Hydrogenated soybean oil	27	1	5
Crude soybean oil	111	6	4
Lard	65	2	4
Milk fat (butter oil)	35	8	10
Milk fat (whole milk)	71	6	12

linolenic and tetraenoic acids, respectively, for 15 calves at 18 days of age. Previously whole milk had been fed at the rate of ten pounds per 100 pounds of body weight. Subsequently, these animals were divided into five lots. During the period from 18 to 46 days one group was restricted to whole milk whereas the others were fed reconstituted milk diets containing various lipids at a three per cent level. The results are shown in Table 13.

E. Role of Essential Fatty Acids in Nutrition

It is well known that careful removal of fat from the diet of a rat will cause fat deficiency syndromes and that the symptoms may be alleviated by appropriate supplementation of the fat-deficient diet. The essential unsaturated fatty acids for the rat are thought to be one or more of the following: linoleic, linolenic and arachidonic acids. Osborne and Mendel (101) in 1920 were the first to propose the dietary essentiality of these unsaturated fatty acids. Preparation of a synthetic diet low enough in fat to prove that fat is a dietary necessity, however, is a recent accomplishment. In 1929 Burr and Burr (26) developed a diet which was practically fat-free. This diet contained highly purified casein, sugar, yeast and mineral salts.

It was found that rats receiving as little as ten drops of lard per day made complete recovery even when extreme fat deficiency symptoms were apparent. An analysis of the composition of the fat soon led to the realization that the unsaturated fatty acids were responsible for the curative effects. A decade later, other investigators confirmed that linoleic, linolenic and arachidonic acids were essential for normal growth, development and health of the rat (77, 124, 125).

A summary of the most frequent symptoms of unsaturated fatty acid deficiency in the rat as reported by different workers (25, 26, 43, 70, 116, 124) is as follows: hind legs become scaly and swollen; tail becomes spotted, ridged and finally necrotic; the hair on the back becomes filled with dandruff and is shed; growth is retarded; degeneration of the kidney takes place, allowing passage of blood into the urine. This kidney condition is the probable cause of death in rats in severe cases of deficiency. Fat and water metabolism is disturbed. It has been observed that rats on fat-free diets consume about twice as much water as the heavier control rats but they do not excrete any more urine and their feces are dry. Water appears to be lost through the skin or lungs or both. Although the animals on the fat-free diet weigh less than controls, they consume about as much feed even though they are not more active than normal animals.

Reproductive abnormalities associated with fat deficiency in the rat have been reported by many workers (2, 26, 44, 47, 83, 90, 104, 122, 125). In the female the following manifestations have been observed: decreased rate of ovulation (26, 104, 122, 125), prolonged gestation and occasional resorption of the embryo (2, 47, 90), prolonged labor with excessive hemorrhage (2, 105) and inadequate lactation

(47). It was further observed that the litters were often so weak that they did not survive long even though delivered alive.

Kummerow *et al*. (87) performed caesarian sections on female rats maintained on a fat-free ration and found living young in every animal examined on the twentieth and on the twenty-first days of the testation period, whereas, dead young began to appear in the uterus after the twenty-first day. When corn oil or hydrogenated fat was included in the diets, normal parturition took place. Animals receiving five per cent corn oil had normal lactations and weaned 85 per cent of their young but all the young from animals supplemented with five per cent of a commercial hydrogenated fat died within 72 hours after birth. The animals which had been kept on a lipid-free diet exhibited approximately the same percentage of total lipids in the blood plasma as those which had received supplements of corn oil. The animals which had received corn oil, however, had almost four times more arachidonic acid in their blood plasma than those on the lipid-free diet. The fat-deficient animals appeared to synthesize oleic acid in a vain attempt to increase the percentage of unsaturated fatty acids in body fat. The mature animals on the fat-deficient diet contained 38.9 per cent more oleic acid (in body fat) than those on corn oil.

The body fat of young from animals which had received corn oil contained from five to ten times more arachidonic acid than that of animals on the fat-free diet, and twice as much arachidonic acid as that of the animals on hydrogenated fat. Arachidonic acid is concentrated in the phospholipid fraction.

These studies suggest that a dietary source of unsaturated fat such as corn oil, is necessary for normal reproduction and lactation. Moreover, commercial hydrogenated fat apparently does not furnish a sufficient amount of essential unsaturated fatty acids to meet the requirements for normal lactation.

Quackenbush et al. (104) found that reproductive failure always developed in female rats which had grown to maturity on a fat-free diet. The gestation period was normal and up to 83 per cent of the young were weaned, however, when the animals were supplemented with as little as 100 mg. ethyl linoleate daily during the three weeks preceding breeding. The results of these investigations indicate that linoleic and arachidonic acids are the limiting factors involved in normal reproduction. It has been postulated that linoleic acid serves as a source of arachidonic acid.

There is some difference of opinion concerning the relative activity of the unsaturated fatty acids. The essential fatty acids seem to differ in both quantitative and qualitative effects. Burr _et al_. (24) have classified linoleic acid as most effective, arachidonic acid intermediate, and linolenic acid least effective with respect to effect on growth. Maynard (94) stated that linoleic acid is of primary importance in the diet because the other two unsaturated acids can be synthesized by the body from it. Turpeinen (129) on the other hand has reported that arachidonic acid is three times as effective as linoleic acid in promoting growth response of the rat. Hume and Nunn (77) stated that arachidonic acid is only twice as potent as linoleic acid in stimulating growth. Greenberg _et al_. (57) recently reported that methyl arachidonate has 3.5 times the biopotency of linoleic acid when fed to fat-deficient rats; the ratio of the biological activity of arachidonate to that of linoleate when these acids were fed as a mixture was found to be 6.2:1.0. Widmer and Holman (131) found the rat could synthesize arachidonic acid from linoleic acid but not from linolenic acid.

It is apparent that requirements for the unsaturated fatty acids are greatly influenced by the other components of the diet. The growth of the rat is greatly retarded and death soon results when carbohydrates are partly replaced by fat completely lacking in the unsaturated fatty acids

(44, 123). The deficiencies were cured either by feeding linoleic acid or by providing a high-carbohydrate diet. Sinclair (123) reported that a larger quantity of linoleic acid is required by rats on a high-fat diet devoid of the essential fatty acids. Deuel et al. (34) also found that when hydrogenated coconut oil was added to a low fat diet of rats their growth rate was depressed. The depressing effect was completely counteracted by adequate supplementation with linoleate. These findings seem to indicate that requirements for linoleic acid are critical in high fat diets. The alleviation of deficiency symptoms by feeding high carbohydrate diets has been explained by assuming that carbohydrates enhance body-synthesis of linoleic acid. The latter hypothesis is debatable since Schoenheimer (118) who used deuterium tagged saturated fatty acids found no indication of deuterium in the linoleic or linolenic acids in the body fat. This finding suggests that linoleic and linolenic acid must be derived from dietary fat and that they are not synthesized. Barki (11) could not confirm these results. He reported that rats maintained on a fat-deficient diet can synthesize linoleic and linolenic acids for rats on the fat-free diet developed deficiency symptoms which subsequently disappeared.

Bacon *et al.* (8) found that if mineral oil at levels below five per cent is added to a low-fat diet, fatty acid deficiencies result and growth ceases in about 12 weeks. When ten per cent mineral oil was added to a low-fat diet, growth failure in the rats occurred in two to three weeks. Fatty acid deficiency symptoms could be prevented by feeding 50 mg. of linoleate per day.

Samuels *et al.* (113) demonstrated a protein-sparing action of high-fat diets. Pearson and Panzer (102) found that the essential fatty acids cause a retention of essential amino acids in the rat.

No absolute values for optimum daily intake of the essential fatty acids for the rat are available. It has been reported (129) that 100 mg. of linoleic or 33 mg. of arachidonic will bring about growth response in the deficient rat. Greenberg *et al.* (57) noted that the gain in weight of rats receiving a cottonseed oil diet was greater than that of rats on a low-fat regimen supplemented with as much as 100 mg. of methyl linoleate or 20 mg. of methyl arachidonate per day. The efficiency of utilization of calories was increased progressively with increasing dosages of essential fatty acids. Greater efficiency in the utilization of the calories was obtained in the males.

Deuel *et al.* (35) found linoleate requirements of female rats on a lipid-free diet to be lower than 20 mg. per day.

The data are not so conclusive in the case of the male. The administration of linoleate at a level of 60 mg. daily did not change the slow rate of increase in body weight of the male rats on the 20 mg. dose, nor did this amount of supplementation result in a stimulation in growth of the female rats which had reached a plateau in growth rate. MacKenzie et al. (90) suggest that 20 mg. daily of linoleate is the minimum level for growth response in rats. Burr et al. (24) suggest that requirements for recovery from the fat deficiency syndrome are higher than 20 mg. of linoleate per day. In contrast Pihl et al. (103), found the requirements for essential fatty acids were met by administering 15 mg. of methyl linoleate per rat per day.

Fat deficiency can be produced in the mouse (19, 130), chicken (108), dog (67) and in certain insects (53). Reviews of these deficiencies have been presented by Burr (23) and Holman (72). Experimentally a frank fat deficiency has not been produced in the human. Nevertheless, much evidence has been accumulated to show similarities between a certain type of infant eczema and fat deficiency. Some cases of infant eczema which did not respond to other treatments improved following the administration of unsaturated fats (66). Other studies of eczema cases in the infant seem to indicate that about one-half of them are related to essential fatty acid deficiencies (48, 74). Hansen (64) found the average iodine number of the serum fatty acids of 11 infants with eczema to be 82, while in 11 normal infants it was 114. Other studies (63, 64, 65) indicate a lower content of arachidonic and linoleic acid in blood plasma of eczematous persons. Improvement was observed in all eczematous infants given a source of essential fatty acids.

Ginsberg et al. (54), found no difference in the serum lipid iodine number between normal patients and those with eczema. These contradictory findings are partially explained by the results of Finnerud et al. (48) who found that only 50 per cent of patients with eczema have subnormal blood lipid iodine numbers. Eczematous patients with normal iodine numbers respond less readily, if at all, to dietary supplementations of unsaturated oils.

It has been found that swine fed low-fat rations produce fat very low in linolenic acid but they do not exhibit fat-deficiency syndromes (40, 41). Witz and Beeson (138) were able to produce a fat deficiency disease in pigs by feeding a ration which was almost lipid-free. The investigation included two experiments involving similar rations containing different kinds of casein. In Experiment I crude casein was fed whereas in Experiment II vitamin test casein was used. The components of the ration used are shown in Table 14.

Table 14

Composition of Lipid-Free Rations Fed to Pigs

Ingredients	Lipid-Free Diet
	(per cent)
Casein	27.0
Lard	0.0
Dextrose	65.8
Salt mixture	5.2
Vitamin supplements	(included 10 of the more common vitamins)
Vitamin B_{12} concentrate	0.5
Liver extract	0.5

Fat-deficiency symptoms were observed in both Experiments but the symptoms in Experiment II were more severe. The lower fat content of the vitamin test casein used in the latter experiment was considered the reason for the increased severity of the symptoms in the animals.

It was noted that fat-deficiency symptoms, which included dandruff, loss of hair and retardation of growth, began to appear after 42 days on the lipid-free diet. After 63 days, the symptoms became quite severe and the pigs had almost stopped growing. Upon addition of 1.5 per cent corn oil to the diet, the pigs made partial recovery. Pigs remaining on the lipid-free diet died after an average of 77 days on the experiment.

Blood analyses revealed that the vitamin A levels were significantly higher in the animals on the lipid-free diet. Level of fat intake apparently had no significant effect on the hemoglobin, cholesterol and red and white cell content of the blood. Blood plasma lipid content, as determined by the "Allen fat" procedure, was significantly higher in the pigs receiving five per cent lard. Feed efficiency of pigs receiving ration in Experiment I was normal but lower for the pigs receiving the vitamin-test casein in Experiment II. Feed efficiency was improved when 1.5 per cent corn oil was incorporated into the fat-free diet.

III. EXPERIMENTAL

A. Management of Calves

Holstein, Brown Swiss and Milking Shorthorn calves were obtained from the Iowa State College Dairy herd during the years 1951-52. Descriptive data relative to these animals are presented in Table 15. During the winter months, the temperature of the calf barn was maintained at about 65° F. by a thermostatically controlled oil furnace. Experimental calves were maintained in individual pens bedded with wood shavings. Each calf was fed 80 mg. of crystalline aureomycin hydrochloride per day. The antibiotic was mixed with lactose to deliver 40 mg. of aureomycin per small deflagrating spoon of mixture and one spoonful of this mixture was stirred into the milk just prior to each feeding.

The only feed given to the calves while on the experiment was the semi-synthetic milk. The amount of milk fed was reduced by one-half the feeding immediately following the detection of any diarrhea and was gradually increased to the regular amount, the rapidity of return to the normal level depending upon the rate of recovery. In severe

Table 15

Descriptive Data Relative to the Experimental Animals

Calf No.	Breed[1]	Sex	Date of Birth	No. of Colostrum Feedings
3596	H	F	8-20-51	3 days *ab libitum*
3597	H	F	8-20-51	3 days *ab libitum*
3603	H	M	9-11-51	0
3640	BS	M	12-10-51	0
3643	BS	M	12-22-51	0
3657	BS	M	1-31-52	1
3671	MS	M	3- 9-52	1
3686	H	M	4- 4-52	1
3688	MS	M	4- 6-52	0
3696	MS	M	4-26-52	1
3697	H	M	4-28-52	1
3698	H	M	5- 6-52	1
3699	H	M	5- 6-52	1
3700	H	M	5-13-52	1
3701	H	M	5-14-52	1
3768	MS	M	9-16-52	1
3773	H	M	10- 7-52	1
3780	H	M	10-20-52	1
3784	H	M	10-28-52	1
3786	BS	M	10-30-52	1

[1]BS-Brown Swiss, H-Holstein, MS-Milking Shorthorn.

cases of diarrhea "Kaopectate"[1] and/or aueromycin at therapeutic levels were administered with warm water. The last five calves used in the experiment, 3768, 3773, 3780, 3784 and 3786, were muzzled from the time they were put into their pens until the termination of the experiment since it was observed that some of the preceding calves consumed shavings.

B. Preparation of Rations

A semi-synthetic milk was used to permit careful control of lipid intake. The composition of this milk, which was similar to the one developed by Clark (30) and later used successfully by Kastelic et al. (84), is presented in Table 16, and the vitamin and salt components are presented in Tables 17 and 18, respectively. Vitamin-test casein was employed as the source of protein in the fat-free diet. This was considered to be justifiable since Deuel et al. (35) found that the fat depletion period of rats receiving vitamin-test casein as their source of protein was about the

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[1]Produced by The Upjohn Company, Kalamazoo, Michigan. This product was a suspension of kaolin combined with pectin. Each fluid ounce contained 90 grs. kaolin and 2 grs. of pectin.

Table 16

Composition of the Semi-synthetic Milk

Components	Per Cent
Casein (vitamin test)[1]	3.5
Fat	0-3.0
Salts[2]	0.2
Lecithin[3]	Variable
Lactose[4]	5.0
Vitamins	See Table 18

[1]Purchased from Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

[2]See Table 17.

[3]Generously supplied by American Lecithin Company, Inc., Woodside, L. I. 77, N. Y.

[4]Generously supplied by Western Condensing Company, Appleton, Wisconsin.

Table 17

Salt Composition of the Semi-synthetic Milk (30)

Component	Quantity per Kg. Milk	Component	Quantity per Kg. Milk
$Ca(OH)_2$	1.200 g.	KOH	0.959 g.
MgO	0.298 g.	NaOH	0.928 g.
KH_2PO_4	2.873 g.	Fe Citrate	2.000 g.
Citric acid	1.998 g.	$MnSO_4 \cdot H_2O$	0.100 mg.
$CaCO_3$	0.140 g.	$CuSO_4 \cdot 5H_2O$	0.500 mg.
HCl (0.1 N)[1]	--	$ZnSO_4 \cdot 7H_2O$	10.000 mg.
$CaCl_2$	1.565 g.	$CoSO_4 \cdot 5H_2O$	1.500 mg.
		KI	0.400 mg.

[1]Variable amounts used to adjust milk to pH 6.6.

Table 18

Vitamin Supplement to Semi-synthetic Milk (84)

Vitamin	Quantity per Calf Daily
Ascorbic acid[1]	150 mg.
Vitamin A[2]	200,000 I. U. first three days
	10,000 I. U. daily from fourth day
Calciferol[3]	500 I. U.
Thiamine[1]	5.0 mg.
Riboflavin[1]	5.0 mg.
Pyridoxine[1]	5.0 mg.
Calcium pantothenate[1]	7.5 mg.
Niacin[1]	14.0 mg.
Inositol[3]	100.0 mg.
Menadione[1]	1.25 mg.
Biotin[3]	0.25 mg.
Pteroylglutamic acid[1]	0.25 mg.
P-aminobenzoic acid[1]	12.50 mg.
Choline[1]	1.0 g.
alpha-Tocopherol[1]	40.0 mg.
Vitamin B_{12}[1]	19 micrograms

[1]Generously supplied by Merck and Co., Rahway, N. J.

[2]Generously supplied by Charles Pfizer and Co. Inc., Terre Houte, Indiana.

[3]Purchased from Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

same as that reported by Burr and Burr (26) and Brown et al. (22) using other highly purified constituents. Deuel et al. (35) also reported that further precipitation of the vitamin-test casein or prolonged extraction of the casein with ethyl alcohol and diethyl ether, or both procedures, failed to shorten the fat-depletion time of the rat.

The semi-synthetic ration was mixed as described by Clark (30) except that 0.1 N HCl was substituted for 0.1 N H_2SO_4 in the final adjustment of the pH to 6.6 (this modification being suggested by Kastelic (84)). The milk then was transferred to ten-gallon milk cans and stored in a cooler maintained at 40° F. until time for feeding. The milk was made every two to three days depending upon the number of calves in the experiment. Twenty to 30 gallons of milk were made at one time.

Just prior to feeding, the milk was warmed to 100° F. and vitamins[1] were measured in a small deflagrating spoon

[1]All of the vitamins except vitamin E and choline were combined in C. P. lactose, which was used as a filler. Vitamin E was the only vitamin not administered in crystalline form. The vitamins were mixed at three to four-week intervals and were refrigerated at 40° F. until fed. Vitamin E and choline were fed at each A. M. feeding only.

and added to the milk. When fat or lipid fractions were used, they were added to the milk and the mixture was homogenized at 3,000 pounds pressure in a Gaulin single-stage homogenizer.

C. Dietary Groups

The Committee on Animal Nutrition of the National Research Council (89) recommends the feeding of two pounds of total digestible nutrients per day per 100 pounds of body weight to dairy calves. To meet these recommendations about 13 pounds of milk containing three per cent fat would be required per 100 pounds of body weight per day (95). However, Wing (134) reported slow growth of calves, as measured by increase in body weight, when feeding reconstituted skim milk (ten per cent non-fat dry milk solids) at the rate of 13 pounds per 100 pounds of body weight per day, and more rapid growth (48 pounds weight gain in 56 days) when the rate was increased to 16 pounds per 100 pounds of body weight.

Preliminary work seemed to indicate that calves could not consume more than 16 per cent of their body weight in milk per day without considerable diarrhea. It was decided, therefore, that calves receiving the fat-free semi-synthetic milk would be fed at the 15 per cent level. It seemed logical to expect a rate of gain at the 16 per cent level similar to that reported by Wing (134), if nutritional deficiencies did not develop.

Hydrogenated soybean oil was fed to some of the calves at two levels of intake. Group IV received 13 pounds of semi-synthetic milk containing three per cent fat, per 100 pounds of weight, thereby approaching the caloric requirements of the calf as recommended by the National Research Council (89). Calves receiving such intake would be expected to approach Ragsdale's growth standards for calves if the diet was nutritionally adequate in other respects. Calves in Groups II and III were fed 11 per cent semi-synthetic milk containing 2 and 3 per cent fat, respectively, per 100 pounds of body weight. Caloric intake of the calves in Group II approached that of calves receiving fat-free semi-synthetic milk at the 16 per cent level. Rations fed during the experimental period are indicated in Table 19. The compositions of the methyl esters, the free fatty acid mix and the pork liver residue are found in Tables 20, 21 and 22, respectively.

Table 19 Dietary Regimes for the Various Experimental Groups

Group	Sub-group	Calf No.	Age (in weeks)	Semi-synthetic Milk[1]	Addition to Basal Semi-synthetic Milk							
					Per Cent in Milk					Grams per Day		
					Soybean Oil		Butter Oil	Lecithin		Methyl Esters[2]	Free Fatty Acids[3]	Pork Liver Residue[4]
					Hydrogenated	Crude		Crude	Purified			
I		3596	0- 8	16	-	-	-	-	-	-	-	-
			8-11	11	2	-	-	-	-	-	-	-
			11-12	11	-	-	2	-	-	-	-	-
		3597	0- 8	16	-	-	-	-	-	-	-	-
			8-11	16	-	-	-	-	-	-	-	-
			11-14	11	-	-	2	-	-	-	-	-
			14-16	11	-	-	2	2	-	-	-	-
		3603	0- 8	16	-	-	-	-	-	-	-	-
			8-13	13	-	1.5	-	-	-	-	-	-
			13-15	11	-	1.5	-	.5	-	-	-	-
		3699	0- 8	16	-	-	-	-	-	-	-	-
			8-12	16	-	-	-	-	-	-	-	-
			12-17	11	-	2.0	-	-	-	-	-	-
			17-19	11	-	1.8	-	-	.2	-	-	-
			19-21	11	1.8	-	-	-	.2	-	-	-
		3700	0- 8	16	-	-	-	-	-	-	-	-
			8-12	16	-	-	-	-	-	-	-	-
			12-17	11	2	-	-	-	-	-	-	-
			17-21	11	1.8	-	-	-	.2	-	-	-
		3768	0- 8	16	-	-	-	-	-	-	-	-
			8-12	16	-	-	-	-	.2	-	-	-
			12-15	13	2.8	-	-	-	.2	-	-	-
		3773	0- 8	16	-	-	-	-	-	-	-	-
			8-12	16	-	-	-	-	-	5.0	-	-
			12-14	16	-	-	-	-	-	15.0	-	-
		3780	0- 8	16	-	-	-	-	-	-	-	-
			8-12	16	-	-	-	-	-	-	-	75±15 g. fat[5]
II		3657	0- 8	11	1.8	-	-	.2	-	-	-	-
		3643	0- 8	11	1.8	-	-	.2	-	-	-	-
		3671	0-12	11	1.8	-	-	-	.2	-	-	-
III	A	3686	0-12	11	3.0	-	-	-	-	-	-	-
		3698	0-12	11	3.0	-	-	-	-	-	-	-
	B	3701	0-12	11	2.8	-	-	-	.2	-	-	-
IV	A	3696	0-12	13	3.0	-	-	-	-	-	-	-
	B	3697	0-12	13	2.8	-	-	-	.2	-	-	-
	C	3688	2-12	13	-	-	3	-	-	-	-	-
V	A	3640	1- 5	16	-	-	-	.2	-	-	-	-
	B	3786	0- 5	16	-	-	-	-	-	-	5	-
			5- 6	16	-	-	-	-	-	-	15	-
			6- 9	13	2.8	-	-	-	.2	-	5	-
	C	3784	0- 5	16	-	-	-	-	-	-	5	-
			5- 8	16	-	-	-	-	-	-	15	-
			8-12	11	1.8	-	-	-	-	-	15	-

[1] Per cent of body weight daily.
[2] Approximately 50% methyl linoleate.
[3] Generously supplied by Emery Industries Inc., Cincinnati, Ohio.
[4] Generously supplied by Armour & Co., Chicago, Illinois.
[5] Protein reduced in semi-synthetic milk by 57.25 gm. per day since this amount of protein was added in the pork liver residue each day.

Table 20

Composition of Methyl Esters

Batch No.	Fraction Fed	Iodine No.[1]	Fatty Acids		
			Linoleic	Linolenic	Arachidonic
			(per cent)		
I	F_3	147.0	68.85	0.0	0.0
II	F_3	119.6	47.67	0.0	0.0
	F_4	114.4	47.39	0.0	0.0
III	F_3	115.3	48.21	0.0	.75
	F_4	122.8	49.84	0.24	0.0
IV	F_4	118.5	51.84	0.0	0.0

[1]Micro Rosenmund-Kuhnhenn method.

Table 21

Composition of the Free Fatty Acid Mixture

Fatty Acids	Per Cent
Linoleic	56.41
Linolenic	0.00
Arachidonic	0.58
Oleic	40.00
Palmitic	3.00
Myristic	1.00

Table 22

Composition of the Pork Liver Residue

Total Fatty Acids	Per Cent
Free fatty acids	29.7
Phospholipid fatty acids	53.5
Neutral fat fatty acids[1]	16.1
Cholesterol ester fatty acids[1]	0.7

Iodine Number and Polyunsaturated Fatty Acid Values

Lipid	Iodine No.[2]	Per Cent		
		Linoleic	Linolenic	Arachidonic
Alcohol-ether soluble	71.9	11.7	0	12.7
Skellysolve A soluble	79.0	10.7	0	11.0
Phospholipid	78.5	14.7	0	16.7
Total fatty acids	105.3	17.0	0	18.8
Free fatty acids	121.9	19.3	0	17.8
Phospholipid fatty acids	123.5	20.5	0	29.0

[1]Determined by calculation.

[2]Micro Rosenmund-Kuhnhenn method.

D. Records

The recorded data included body weight changes, incidence of diarrhea, amount of milk consumed, deficiency symptoms and blood plasma analyses. Venous blood samples were taken from each animal at weekly intervals and the plasma was analyzed for "Allen fat" and total fatty acids. In addition blood plasma levels of phospholipids and linoleic, linolenic and arachidonic fatty acids were determined for most of the calves. The calves were weighed when taken from their dams and daily thereafter, just prior to the evening feeding.

Photographs were taken of a number of the calves, some of which have been included in this thesis.

E. Chemical Methods

At weekly intervals 100 ml. of blood samples were obtained from the jugular vein of the calves approximately three hours after feeding. Heparin was used as the anticoagulant.

Lipids were extracted from an aliquot of plasma (varying from 15 to 30 ml. depending upon lipid content) with alcohol-ether (3:1) using a solvent to plasma ratio of 20 to 1. The plasma was added dropwise to the alcohol-ether with constant agitation. The mixture was heated to a gentle boil for a few seconds over a hot water bath and then was cooled. The heating was repeated, then the mixture was filtered through a pyrex sintered-glass filter of medium porosity to remove the precipitated protein.

The alcohol-ether extract was evaporated to four to five ml. volume under reduced pressure (water pump) on a water bath at a temperature of 55-60° C. The concentrated extract was saponified directly over a hot plate (maintained at 65-75° C.) for one hour, employing a modification of the procedure of Boyd (20). Two-tenths ml. of saturated aqueous NaOH (43 grams/100 ml.) to each four ml. of plasma represented in the extract was used for saponification. Evaporation was controlled so that at the end of one hour three to four ml. of solution remained. Additions of alcohol-ether were made during saponification to prevent evaporation below this volume. The saponification mixture was quantitatively transferred into a glass-stoppered 50 ml. centrifugation tube, washing the saponification flask three times with three to four ml. of water and then three or four times with Skellysolve A. Liberation of the fatty acids was accomplished by adding two drops of 0.1 per cent phenol red indicator and then 25 per cent H_2SO_4 dropwise to the mixture until the aqueous layer turned yellow. After 10 to 15 minutes the mixture was extracted three times with Skellysolve A. The extracts were combined and brought to volume in a 100 ml. volumetric flask. This solution contained the total fatty acids and the total cholesterol and other non-saponifiable components.

The total fatty acids were separated from the non-saponifiable material by employing an alcoholic alkaline wash as described by Wilson and Hansen (133). The total fatty acids were extracted, after acidification, in Skellysolve A and brought to volume in a 100 ml. volumetric flash. Aliquots of this material were in turn used for micro-oxidative analysis as described by Bloor (18) and Boyd (20) for the determination of the total fatty acids. Finally, aliquots were taken for estimation of the plasma polyunsaturated fatty acids by alkali isomerization.

Isomerization was accomplished by heating the fatty acids with potassium hydroxide-ethylene glycol reagent for 30 minutes at 180° C. under an atmosphere of nitrogen. The potassium hydroxide-ethylene glycol reagent was prepared as described by O'Connor _et al_. (100). A Beckman spectrophotometer, Model DU, was employed to measure optical densities at wavelengths recommended by Brice and Swain (21) for estimating linoleic, linolenic and arachidonic acids. The background absorption for the unisomerized fatty acids dissolved in isooctane indicated that essentially no conjugated compounds were present in the original fatty acid aliquots. The spectral data were converted to the plasma levels of linoleic, linolenic and arachidonic acids by the method described by Brice and Swain (21).

The "Allen fat" values were determined on each plasma sample using the procedure developed by Allen (3). Another 0.5 ml. portion of plasma was used for the microdetermination of plasma phospholipids as described by Zilversmit and Davis (141).

All chemical determinations were run in duplicate and the average values were used. In a few instances where obvious errors occurred the analysis was repeated.

IV. RESULTS

In studying the problem of the lipid requirements of the young dairy calf, it was decided to feed one group of calves (Group I) a lipid-free diet and subsequently to reduce the intake of semi-synthetic milk and add a lipid or lipid fractions to the diet. Other calves (Groups II, III, IV and V) with the exception of one calf received the semi-synthetic milk plus lipids or lipid fractions from birth. The diets of calves in Group II were essentially iso-caloric to the diet fed to Group I. Criteria used for evaluating the various dietary regimens were (a) external appearance; (b) body weight changes; (c) efficiency of feed utilization; and (d) blood plasma values for "Allen fat", phospholipids, total fatty acids and linoleic, linolenic and arachidonic acids.

A. External Manifestations of Fat Deficiency

Manifestations of fat deficiency first were observed in one calf after being on the lipid-free diet for 42 days. At 56 days the syndromes were quite severe (Figure 1) in about

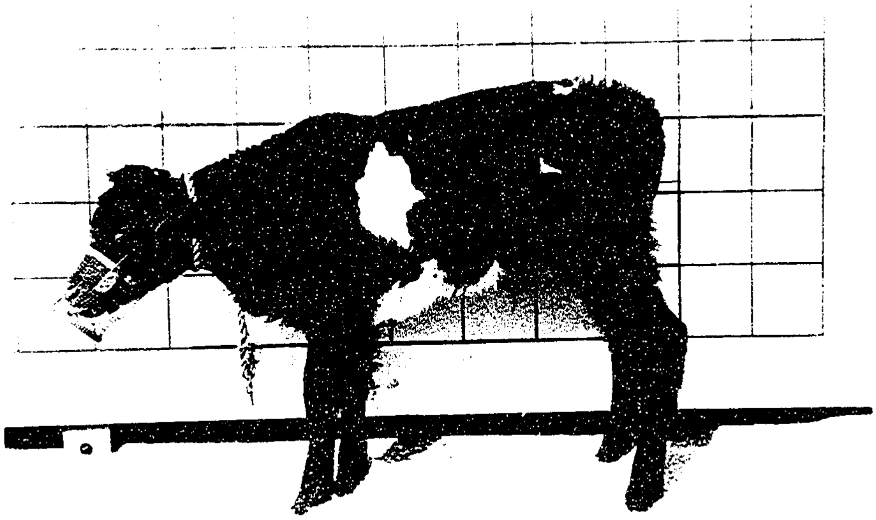

Figure 1. Male calf 3768 exhibiting fat deficiency symptoms at 56 days of age

50 per cent of the animals. Scaly dandruff appeared over all of the body but was more concentrated across the shoulders, along the back and tail. Another symptom was the loss of part of the hair over the back, shoulders and tail. The hair remaining on the body was long, dry and lacked the luster of the hair on calves receiving fat. Alopecia was occasionally observed on the neck and tail of the calves on the lipid-free diet.

The incidence of diarrhea in all experimental calves (Table 23) was comparatively high during the second week. Except for Group V the incidence of diarrhea was low from the second to the eighth week in all groups receiving lipid supplementation. Diarrhea became more common as calves on the lipid-free milk approached 56 days and it became increasingly more difficult to cure cases of diarrhea by treatment procedures previously discussed. The calves in Group V suffered from an excessive amount of diarrhea. Two of the calves, 3784 and 3786, received about 0.2 per cent free fatty acids while calf 3640 received lecithin in the semi-synthetic milk. These lipids fed singly in the synthetic milk seemed to increase the incidence of diarrhea, whereas, when they were fed in combination with other fats, diarrhea was not excessive.

Table 23

Effect of Dietary Regimen on Incidence of Diarrhea in Young Dairy Calves

Dietary Group	Sub-group	Calf No.	Weeks on Experiment 1	2	3	4	5	6	7	8
			(number of days diarrhea occurred)							
I		3596	0	0	0	2	0	0	1	2
		3597	0	1	0	0	0	0	0	1
		3603	0	0	2	0	0	0	3	4
		3768	0	2	2	0	0	0	3	6
		3780	0	3	0	0	4	0	0	2
		3773	0	0	0	2	0	0	1	2
		3699	0	1	0	0	0	0	1	1
		3700	1	2	0	0	1	0	2	4
Average			0.1	1.1	0.5	0.5	0.6	0.0	1.4	2.8
II		3657	1	1	0	0	0	0	0	1
		3643	0	1	0	0	0	0	0	0
		3671	2	1	0	0	0	0	0	0
Average			1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.3
III	A	3686	4	0	2	0	0	0	0	0
		3698	1	3	2	0	0	0	0	0
	B	3701	3	3	0	0	0	1	0	0
Average			2.7	2.0	1.3	0.0	0.0	0.3	0.0	0.0

Table 23 (Cont'd)

Dietary Group	Sub-group	Calf No.	Weeks on Experiment							
			1	2	3	4	5	6	7	8
IV	A	3696	3	0	0	0	2	0	0	0
	B	3697	0	0	1	0	0	0	0	0
	C	3688	0	4	0	0	0	0	0	0
Average			1.0	1.3	0.3	0.0	0.7	0.0	0.0	0.0
V	A	3640	3	4	5	5	7	0	1	0
	B	3786	1	7	3	5	6	7	0	0
	C	3784	0	3	0	5	3	2	0	0
Average			1.3	4.7	2.7	5.0	5.3	3.0	0.3	0.0

After 56 days, five calves (Group I) were given various lipids or lipid fractions, the lipids replacing lactose and casein to maintain an iso-caloric intake per 100 pounds of body weight. Following this treatment, the dandruff condition was alleviated, the skin became more oily and a smooth hair coat began to appear. The calves seemed to become more alert, the loss of hair ceased and new hair began to grow in areas previously showing alopecia. Calves receiving butter oil or hydrogenated soybean oil plus lecithin seemed to recover more rapidly than those fed hydrogenated soybean oil, crude soybean oil or lecithin. Fair response to methyl esters and pork liver fat was observed, although the responses were not so spectacular as when butter oil or hydrogenated soybean oil plus lecithin were fed. Three calves, one female and two males, which seemed to be the healthiest animals in Group I at 56 days were continued on the lipid-free ration to 84 days.

B. Body Weight Changes

The weekly body weights for all calves are presented in Table 24. Calves in Group I (lipid-free ration) did not lose so much weight initially as calves which received lipids. Weight gains of calves in Groups II, III and IV greatly

Table 24 Effect of Dietary on Changes in Body Weight

Dietary Group	Sub-group	Calf No.	Breed	Sex	Ration: Syn. Milk[1]	Ration: Lipid	Age in Days: 0	7	14	21	28	35	42	49 (lbs.)	56	63	
I		3596	H	F	16	None	65.0	67.0	72.0	73.0	71.0	69.0	72.0	73.0	72.5		
					11	2% H.S.B.O.										73.5	7
					11	2% B.O.											
		3597	H	F	16	None	73.0	76.0	82.0	83.0	80.0	84.0	86.0	86.5	85.0	85.0	8
					11	2% B.O.											
					11	2% B.O. + 2% Lec. (crude)											
		3603	H	M	16	None	95.0	97.0	97.0	98.5	96.0	96.5	93.5	96.5	95.0		
					13	1.5% C.S.B.O.										95.0	9
					11	1.5% C.S.B.O. + .5% Lec.											
		3768	MS	M	16	None	69.5	69.5	70.0	71.0	74.0	77.0	79.5	82.5	84.5		
					16	7.5 g. Lec.										85.0	8
					13	2.8% H.S.B.O. + .2% Lec.											
		3780	H	M	16	None	71.0	71.0	72.5	71.0	74.5	78.0	76.0	78.0	78.0		
					16	Pork Liver ≈ 15 g. Fat										82.0	8
		3773	H	M	16	None	80.0	79.0	79.5	83.0	87.5	86.0	90.0	91.5	92.0		
					16	5 g. Methyl Esters										93.0	9
					16	15 g. Methyl Esters											
		3699	H	M	16	None	74.0	73.5	75.0	77.0	79.5	81.5	84.5	92.0	92.5	96.0	9
					11	2% C.S.B.O.											
					11	1.8% C.S.B.O. + .2% Lec.											
					11	1.8% H.S.B.O. + .2% Lec.											
		3700	H	M	16	None	88.0	87.0	88.5	87.0	90.0	94.0	100.0	102.5	103.0	106.5	10
					11	2% H.S.B.O.											
					11	1.8% H.S.B.O. + .2% Lec.											
Average 0-8 Weeks on Fat-free Ration							76.9	77.5	79.6	80.4	81.6	83.3	85.2	87.8	87.8		
Average 8-12 Weeks on Fat-free Ration																96.2	9
II		3657	BS	M	11	1.8% H.S.B.O. + .2% Lec.	120.0	121.0	120.5	125.0	132.5	139.0	145.5	154.5	157.0		
		3643	BS	M	11	1.8% H.S.B.O. + .2% Lec.	103.0	103.0	99.0	101.0	108.0	110.0	115.0	121.0	125.0		
		3671	MS	M	11	1.8% H.S.B.O. + .2% Lec.	92.5	93.5	95.0	96.0	99.0	106.0	113.0	120.0	124.5	132.5	13
Average							105.2	105.8	104.8	107.3	113.2	118.3	124.5	131.8	135.5		
III	A	3686	H	M	11	3% H.S.B.O.	112.5	111.0	104.5	102.0	102.5	110.0	117.5	121.0	128.0	132.5	13
		3698	H	M	11	3% H.S.B.O.	120.0	123.0	124.0	123.5	127.0	130.0	138.0	146.5	151.5	157.0	15
Average							116.2	117.0	114.2	112.7	114.7	120.0	127.7	133.7	139.7	144.7	14
	B	3701	H	M	11	2.8% H.S.B.O. + .2% Lec.	97.0	96.0	94.0	98.0	102.0	109.0	117.5	122.5	129.5	134.5	13
IV	A	3696	MS	M	13	3% H.S.B.O.	88.5	87.0	89.0	92.5	96.5	100.0	104.0	111.0	116.0	124.0	13
	B	3697[2]	H	M	13	2.8% H.S.B.O. + .2% Lec.	88.0	85.0	87.5	89.0	95.5	101.5	107.5	114.5	121.5	132.5	13
	C	3688[2]	MS	M	13	3% B.O.	80.0	77.0	72.0	80.0	86.5	94.5	104.0	113.5	122.5	129.0	13
Average							85.5	83.0	82.8	87.2	92.8	98.7	105.2	113.0	120.0	128.5	13
V	A	3640[3]	BS	M	16	5 g. Lec.	82.0	77.5	73.0	65.0	68.0	64.5					
	B	3786	BS	M	16	5 g. F.F.A.	98.0	97.0	86.0	86.0	86.0	83.0					
					16	15 g. F.F.A.							80.0				
					13	2.8% H.S.B.O. + .2% Lec.								91.0	96.0	106.0	
	C	3784	H	M	16	5 g. F.F.A.	77.0	74.0	78.0	78.0	81.5	83.0					
					16	15 g. F.F.A.							84.0	88.5	92.0		
					11	1.8% H.S.B.O. + .2% Lec.										97.5	10

[1] Per cent of body weight daily.
[2] On fat-free ration first two weeks, at rate of 16 per cent of body weight.
[3] Taken off experiment to save life.

Dietary on Changes in Body Weight

Age in Days																				
7	14	21	28	35	42	49 (lbs.)	56	63	70	77	84	91	98	105	112	119	126	133	140	147
67.0	72.0	73.0	71.0	69.0	72.0	73.0	72.5													
								73.5	74.0	72.5										
											74.0	81.0								
76.0	82.0	83.0	80.0	84.0	86.0	86.5	85.0	86.0	87.5	86.0	85.5									
												84.0	77.5							
														83.0	85.0					
97.0	97.0	98.5	96.0	96.5	93.5	96.5	95.0													
								95.0	95.0	97.0	100.5	101.5								
													104.0	107.0						
69.5	70.0	71.0	74.0	77.0	79.5	82.5	84.5													
								85.0	85.5	83.5	84.0									
												92.5	98.0	108.0						
71.0	72.5	71.0	74.5	78.0	76.0	78.0	78.0													
								82.0	87.0	95.0	100.0									
79.0	79.5	83.0	87.5	86.0	90.0	91.5	92.0													
								93.0	99.5	100.0	101.0									
												106.0	110.0							
73.5	75.0	77.0	79.5	81.5	84.5	92.0	92.5	96.0	95.0	99.0	104.0									
												106.0	110.5	109.5	113.0	114.5				
																	117.0	124.0		
																			125.5	134.5
87.0	88.5	87.0	90.0	94.0	100.0	102.5	103.0	106.5	107.5	114.5	116.0									
												122.0	121.5	123.5	128.5	135.0				
																	143.5	149.5	157.5	165.5
77.5	79.6	80.4	81.6	83.3	85.2	87.8	87.8													
								96.2	96.7	99.8	101.8									
121.0	120.5	125.0	132.5	139.0	145.5	154.5	157.0													
103.0	99.0	101.0	108.0	110.0	115.0	121.0	125.0													
93.5	95.0	96.0	99.0	106.0	113.0	120.0	124.5	132.5	138.0	145.5	155.0									
105.8	104.8	107.3	113.2	118.3	124.5	131.8	135.5													
111.0	104.5	102.0	102.5	110.0	117.5	121.0	128.0	132.5	137.5	145.0	151.0									
123.0	124.0	123.5	127.0	130.0	138.0	146.5	151.5	157.0	158.0	159.0	165.5									
117.0	114.2	112.7	114.7	120.0	127.7	133.7	139.7	144.7	147.7	152.0	158.2									
96.0	94.0	98.0	102.0	109.0	117.5	122.5	129.5	134.5	137.0	144.5	154.5									
87.0	89.0	92.5	96.5	100.0	104.0	111.0	116.0	124.0	131.5	132.5	138.0									
85.0	87.5	89.0	95.5	101.5	107.5	114.5	121.5	132.5	137.0	141.0	150.0									
77.0	72.0	80.0	86.5	94.5	104.0	113.5	122.5	129.0	136.5	145.0	154.0									
83.0	82.8	87.2	92.8	98.7	105.2	113.0	120.0	128.5	135.0	139.5	147.3									
77.5	73.0	65.0	68.0	64.5																
97.0	86.0	86.0	86.0	83.0																
					80.0															
						91.0	96.0	106.0												
74.0	78.0	78.0	81.5	83.0																
					84.0	88.5	92.0													
								97.5	101.5	106.0	114.0									

ght.

exceeded those of calves in Group I after about two weeks. The mean total weight gain from 0 to 56 days was 10.9 pounds for calves fed the lipid-free diet as compared to 30.3 pounds for those (Group II) that received an iso-caloric diet which contained 1.8 per cent hydrogenated soybean oil and 0.2 per cent lecithin. The mean weight change of calves on the lipid-free diet (Group I) was zero during the period between 49 and 56 days.

Calves in Groups III and IV were fed at a higher caloric intake (than those in Groups I and II) to provide observations on weight gains at higher caloric levels. Calves in Group III received semi-synthetic milk which contained three per cent fat, at the rate of 11 per cent of body weight daily. Calves in Group IV were fed similarly to Group III except that they received milk at the rate of 13 per cent of their body weight daily. Although only one calf was placed in each sub-group in Group IV, response of 3688 is of particular interest. This calf received lipid-free milk for two weeks and lost weight during most of this period. The semi-synthetic milk intake then was reduced from 16 to 13 per cent of his body weight daily and three per cent butter oil was added. This calf responded well and the average weight gains were slightly over a pound a day from the second week until the termination of the experimental period (84 days).

Calves in Group V received free fatty acids or hydrogenated soybean oil and/or lecithin in a semi-synthetic milk. Calf 3640 received purified lecithin but lost weight continually (probably due to excessive diarrhea) and it was removed from the experiment to save its life. The two calves in sub-groups B and C received small amounts of a mixture of free fatty acids which were homogenized into the semi-synthetic milk before each feeding. Excessive diarrhea caused the calf in sub-group B to lose 15 pounds during the first five weeks. The quantity of milk then was reduced and hydrogenated soybean oil and lecithin were added to the ration. Rate of recovery was spectacular and the calf gained 11 pounds during the subsequent week and a total of 26 pounds during the 21-day period of fat supplementation. Calf 3784 in sub-group C displayed a high incidence of diarrhea and showed erratic weight changes during the first eight-week period while free fatty acids were fed. Growth rate appeared to be accelerated from the eighth to the twelfth week period when it was given hydrogenated soybean oil and lecithin.

The changes in weight of the individual calves at various intervals of time are summarized in Table 25. In the first period, from 0-6 weeks, calves on the lipid-free ration gained only 8.3 pounds whereas animals (Group II) on

Table 25 Summary of Effect of Dietary on Changes in Body Weight

Dietary Group	Sub-group	Calf No.	Breed	Sex	Ration: Syn. Milk[1]	Ration: Lipid	0-6 Week	6-8 Week	8-12 Week	12-15 Week	15-17 Week	17-19 Week	19-21 Week
							(weight change, lbs.[2])						
I		3596	H	F	16	None	7.0	0.5					
					11	2% H.S.B.O.			1.5				
					11	2% B.O.				7.0(13)			
		3597	H	F	16	None	13.0	-1.0	0.5				
					11	2% B.O.				-2.5			
					11	2% B.O. + 2% Lec.					2.0(16)		
		3603	H	M	15	None	-1.5	1.5					
					13	1.5% C.S.B.O.			5.5				
					11	1.5% C.S.B.O. + .5% Lec.				1.0			
		3708	MS	M	16	None	10.0	5.0					
					15	7.5 g. Lec.			-0.5				
					13	2.8% H.S.B.O. + .2% Lec.				24.0			
		3780	H	M	16	None	5.0	2.0					
					16	Pork Liver ⇔ 15 g. Fat			22.0				
		3773	H	M	16	None	10.0	2.0					
					16	5 g. Methyl Esters			9.0				
					15	15 g. Methyl Esters				9.0(14)			
		3699	H	M	16	None	10.5	8.0	11.5				
					11	2% C.S.B.O.				5.5	5.0		
					11	1.8% C.S.B.O. + .2% Lec.						9.5	
					11	1.8% H.S.B.O. + .2% Lec.							10.5
		3700	H	M	15	None	12.0	3.0	13.0				
					11	2% H.S.B.O.				7.5	11.5		
					11	1.8% H.S.B.O. + .2% Lec.						14.5	16.0
Average of Fat-free Group							8.3	2.6	8.3				
II		3657	BS	M	11	1.8% H.S.B.O. + .2% Lec.	25.5	11.5					
		3643	BS	M	11	1.8% H.S.B.O. + .2% Lec.	12.0	10.0					
		3671	MS	M	11	1.8% H.S.B.O. + .2% Lec.	20.5	11.5	30.5				
Average							19.3	11.0	30.5				
III	A	3686	H	M	11	3% H.S.B.O.	5.0	10.5	23.0				
		3698	H	M	11	3% H.S.B.O.	18.0	13.5	14.0				
Average							11.5	12.0	18.5				
	B	3701	H	M	11	2.8% H.S.B.O. + .2% Lec.	20.5	12.0	25.0				
IV	A	3696	MS	M	13	3% H.S.B.O.	15.5	12.0	22.0				
	B	3697	H	M	13	2.8% H.S.B.O. + .2% Lec.	19.5	14.0	28.5				
	C	3588[3]	MS	M	13	3% B.O.	24.0	18.5	31.5				
V	A	3640	BS	[illegible]	15	5 g. Lec.	-17.5(5)						
	B	3786[4]	BS	M	15	5 g. F.F.A.	-17.0						
					13	2.8% H.S.B.O. + .2% Lec.		10.0	10.0(9)				
	C	3784[4]	H	M	16	5 g. F.F.A.	7.0						
					16	15 g. F.F.A.		8.0					
					11	1.8% H.S.B.O. + .2% Lec.			22.0				

[1] Per cent of body weight daily
[2] Number in parenthesis indicates last week calf was on experiment.
[3] On fat-free ration for first two weeks, at rate of 15 per cent of body weight.
[4] Received 15 g. F.F.A. during the sixth week.

an iso-caloric intake gained 19.3 pounds. Most of the calves in Groups III and IV made better gains. Calves receiving hydrogenated soybean oil as the only lipid gained less than animals receiving butter oil or lecithin and hydrogenated soybean oil. During the 6-8 week period, calves on the lipid-free ration had a mean weight gain of 2.6 pounds, whereas, calves in Group II made average gains of 11 pounds during this same period and calves in Groups III and IV made still higher gains. The best gain (18.5 pounds) was made by a calf receiving butter oil at the 3 per cent level during the 14-day period. Three calves were maintained on the lipid-free ration for 12 weeks. The weight gain for 3597 and 3700 were somewhat better than weight gains of other calves in the lipid-free group. These two animals were not muzzled and it was observed that they consumed appreciable quantities of wood shavings which were used for bedding. The average weight gain of these three calves on the fat-free ration from 8 to 12 weeks was 8.3 pounds, whereas, weight gains of calves in Groups II, III and IV ranged from 14 to 31.5 pounds during the same period.

The effect of the diet upon the average body weight changes of calves in the various dietary groups is shown in Figure 2. These data indicate the marked difference in growth rates particularly for calves in Groups I and II both

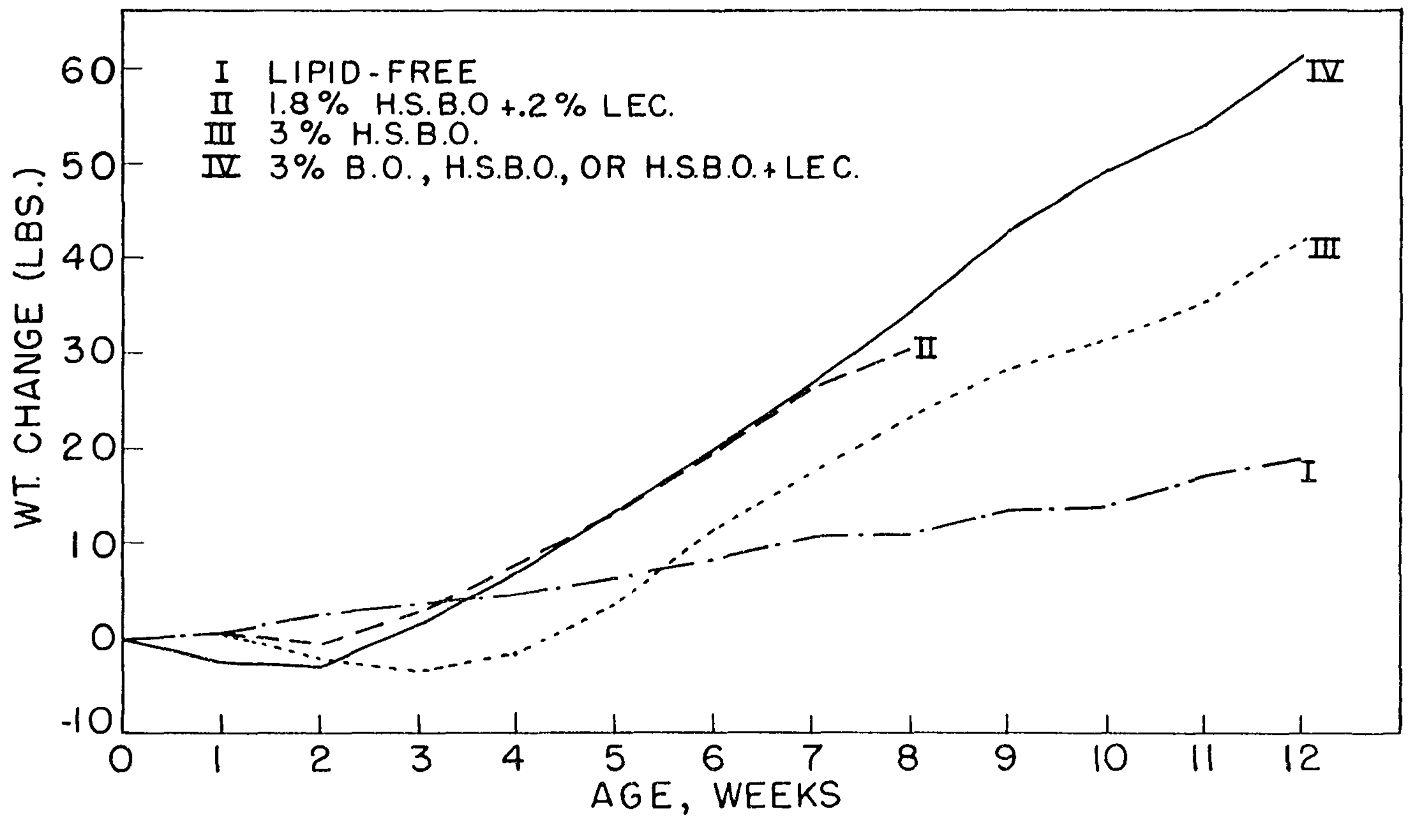

Figure 2. Effect of dietary on changes in group mean weights of calves

of which were fed at an iso-caloric level per 100 pounds of body weight.

After the calves in Group I had received the lipid-free semi-synthetic milk for 8 weeks and for 12 weeks, calves 3597, 3699 and 3700, various lipids were introduced into the diet. These results are indicated in Figure 3. Calf 3597 lost weight and became very weak and emaciated before butter oil was introduced into the ration. Weight loss continued when butter oil was introduced but when the diet was supplemented with lecithin a partial recovery was made. There was no response by 3596 to hydrogenated soybean oil but a partial recovery was made when butter oil was substituted for hydrogenated soybean oil. Animal 3603 responded slowly to crude soybean oil. Calf 3699 also responded slowly to crude soybean oil but the recovery in both appearance and growth was somewhat better when hydrogenated soybean oil plus lecithin replaced the crude soybean oil. A partial recovery was made by 3700 on hydrogenated soybean oil, but the recovery was more spectacular (as determined by appearance and growth) when the fat was supplemented with lecithin.

Calf 3768 did not exhibit any improvement in appearance or increase in weight gain when lecithin was administered yet the recovery was prompt when hydrogenated soybean oil and lecithin were added to the ration. Fifteen grams daily of pork liver fat restored growth and normal appearance to 3680.

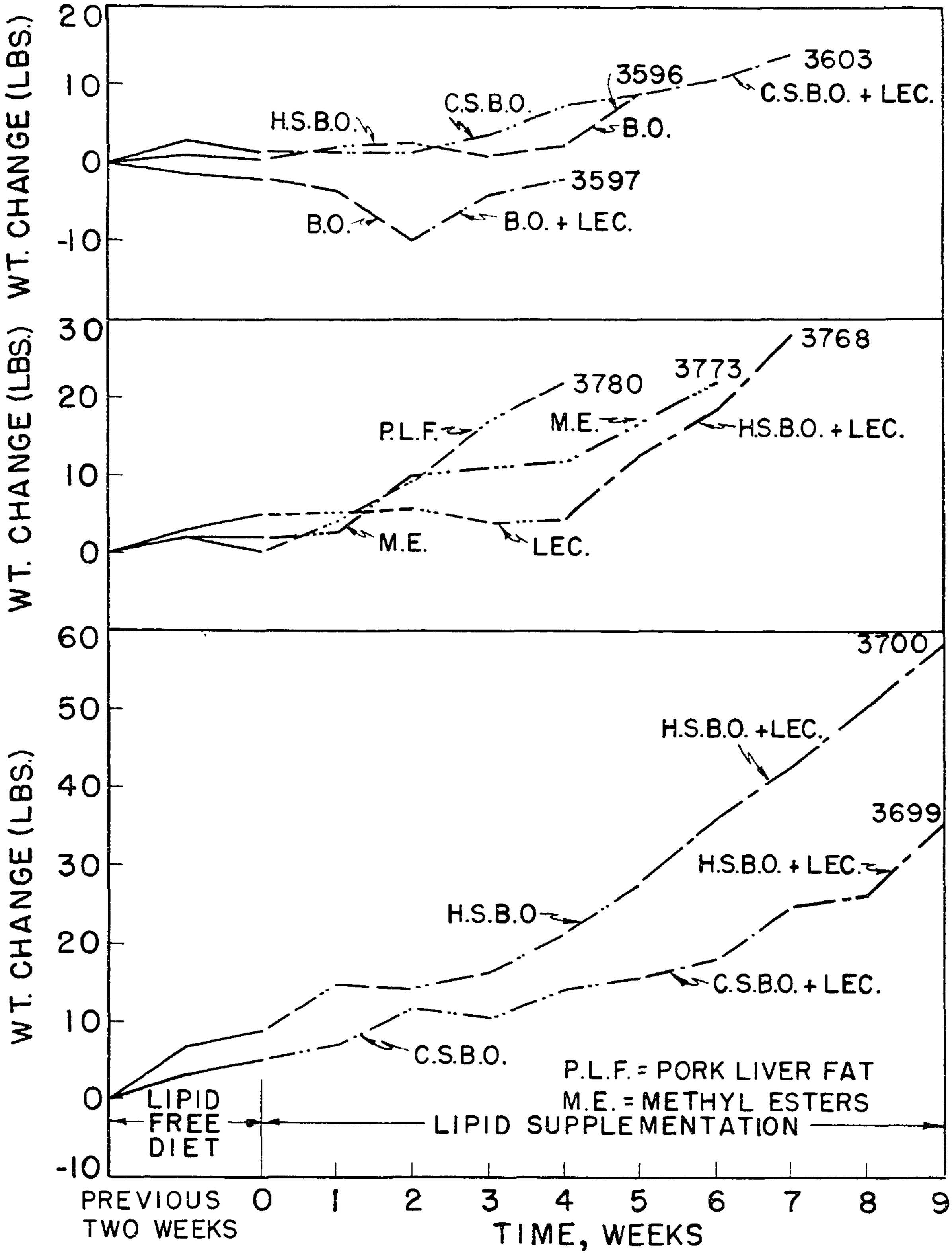

Figure 2. Body weight changes of calves receiving lipids subsequent to lipid-free rations

Calf 3773 responded to methyl esters (approximately 50 per cent methyl-linoleate). The response was slow when five grams of methyl esters were administered per day, but when 15 grams were fed daily the response was greatly improved.

C. Feed Efficiency

The weekly semi-synthetic milk intake of calves up to eight weeks is summarized in Table 26. A breakdown of the total lactose, casein and fat consumed by each calf during this period is also included. The digestion coefficients as given by Morrison (95) were used to transform these values into approximate TDN consumption. Table 27 summarizes the average daily gain, average TDN consumed per day, average TDN per 100 pounds of body weight and TDN per pound of gain up to eight weeks of age.

It is noteworthy that the average TDN intake per 100 pounds of body weight for calves in both Groups I and II was 1.2. Calves in Groups III and IV which were on a higher caloric intake consumed 1.5 and 1.7 pounds of TDN per 100 pounds of body weight, respectively. Calves in the latter group were on the highest plane of nutrition, calorically, but were below the two pounds of TDN per 100 pounds of body

Table 26 Lipid and Milk Intake by Calves During the Initial Eight-Week Period

Dietary Group	Sub-group	Calf No.	Breed	Sex	Initial Weight	Age in Days 7	14	21	28	35	42	49	56	Total Milk Consumed	TDN from CHO	Protein	Fat	Total TDN[1]
						(Lbs. milk consumed)												
I		3596	H	F	65.0	41.6	59.5	68.4	80.5	88.4	94.0	89.0	89.0	610.4	29.91	20.08	-	49.99
		3597	H	F	73.0	44.6	62.8	86.9	89.1	99.4	100.8	88.6	88.6	660.8	32.38	21.74	-	54.12
		3603	H	M	95.0	79.8	96.0	102.2	94.6	99.2	103.4	100.4	98.8	774.4	38.09	25.58	-	63.67
		3768	MS	M	69.5	63.9	72.1	76.2	79.3	83.3	86.7	81.9	89.6	633.0	31.02	20.83	-	51.85
		3773	H	M	80.0	63.6	80.4	89.2	88.2	92.1	97.8	102.2	102.2	715.7	35.04	23.55	-	58.59
		3780	H	M	71.0	66.8	68.9	77.7	81.3	84.0	77.2	83.4	86.6	625.9	30.64	20.60	-	51.24
		3699	H	M	74.0	63.3	67.0	68.0	77.0	89.6	93.8	102.2	93.3	654.2	32.05	21.53	-	53.58
		3700	H	M	88.0	76.6	71.0	75.0	85.2	92.6	101.4	111.0	100.8	713.6	34.97	23.48	-	58.45
Average					76.9	62.5	72.2	80.5	84.4	91.1	94.4	94.8	93.6	673.5	33.01	22.17		55.19
II		3657	BS	M	120.0	70.6	74.9	83.9	95.1	102.7	112.7	126.2	117.0	783.1	38.38	25.77	34.18	98.33
		3643	BS	M	103.0	64.0	70.2	72.3	76.8	84.4	86.8	89.6	91.0	635.1	31.12	20.90	27.72	79.73
		3671	MS	M	92.5	60.7	65.1	59.4	69.6	68.7	75.0	85.6	90.3	574.3	28.15	18.89	25.04	72.08
Average					105.2	65.1	70.1	71.9	80.5	85.3	91.5	100.5	99.4	664.3	32.55	21.85	28.98	83.38
III	A	3686	H	M	112.5	50.6	56.0	56.4	67.2	80.6	91.4	91.4	95.0	588.6	28.84	19.36	38.54	86.74
		3698	H	M	120.0	82.6	73.0	75.6	93.8	94.2	100.2	105.5	112.2	737.1	36.12	24.25	48.26	108.63
Average					116.2	66.6	64.5	66.0	80.5	87.4	95.8	98.5	103.6	662.9	32.48	21.81	43.40	97.69
		3701	H	M	97.0	60.1	60.3	65.0	68.6	79.8	84.0	91.0	95.2	604.0	29.60	19.87	39.56	89.03
IV	A	3696	MS	M	88.5	53.4	74.4	82.6	84.6	97.9	92.6	94.2	97.4	677.1	33.08	22.28	44.33	99.69
	B	3697	H	M	88.0	53.4	74.4	82.6	83.6	80.0	91.4	98.4	105.3	669.1	32.79	22.01	43.81	98.61
	C	3688[2]	MS	M	80.0	57.4	49.8	56.8	74.8	82.6	87.5	94.6	100.2	603.7	29.58	19.86	39.53	88.97
V	A	3640[3]	BS	M	82.0	52.4	61.9	69.7	70.0	46.0				300.0	14.70	9.87	0.86	25.43
	B	3786[4]	BS	M	98.0	75.6	56.6	71.9	60.0	71.5	69.5			405.0	19.85	13.33	1.35	34.53
					80.0							76.2[6]	83.2	159.4	7.82	5.25	10.46	23.53
	C	3784	H	M	77.0	64.2	77.9	82.1	65.5	59.9[5]	94.8	97.3	101.6	643.3	31.53	21.17	2.36	55.06

1 Diegestion coefficients used were, protein 94%, lactose 98% and fat 97% as given by Morrison (95).
2 On fat-free diet for first two weeks.
3 Taken off experiment to save life.
4 Received free fatty acids for only six weeks.
5 Free fatty acids increased from 5 to 15 g. per day
6 H.S.B.O. + lecithin added to ration in an attempt to get recovery.

Table 27 Effect of Dietary on Efficiency of Feed Utilization During Initial Eight-Week Period

Dietary Group	Sub-group	Calf No.	Breed	Sex	TDN Consumed	Initial Weight	Weight at 8 Weeks	Total Gain	Av. Daily Gain	Av. TDN Per Calf	Av. TDN Per 100 lb. Body Wt.[1]	TDN per Lb. Gain
								(lbs.)				
I		3596	H	F	49.99	65.0	72.5	7.5	0.134	0.9	1.2	6.7
		3597	H	F	54.12	73.0	85.0	12.0	0.214	1.0	1.1	4.5
		3603	H	M	63.67	95.0	95.0	0.0	0.000	1.1	1.2	No Gain
		3768	MS	M	51.85	69.5	84.5	15.0	0.268	0.9	1.2	3.5
		3773	H	M	58.59	80.0	92.0	12.0	0.214	1.0	1.2	4.9
		3780	H	M	51.24	71.0	78.0	7.0	0.125	.9	1.2	7.3
		3699	H	M	53.58	74.0	92.5	18.5	0.330	1.0	1.1	2.9
		3700	H	M	58.45	68.0	103.0	15.0	0.268	1.0	1.1	3.9
Average					55.19	76.9	87.8	10.9	0.194	1.0	1.2	4.8
II		3657	BS	M	98.33	120.0	157.0	37.0	0.661	1.8	1.1	2.7
		3643	BS	M	79.73	103.0	125.0	22.0	0.393	1.4	1.3	3.6
		3671	MS	M	72.08	92.5	124.5	32.0	0.571	1.3	1.2	2.3
Average					83.38	105.1	135.5	30.3	0.542	1.5	1.2	2.8
III	A	3686	H	M	86.74	112.5	128.0	15.5	0.277	1.6	1.4	5.6
		3698	H	M	108.63	120.0	151.5	31.5	0.563	1.9	1.3	3.5
Average					97.69	116.3	139.8	23.5	0.420	1.8	1.4	4.5
	B	3701	H	M	89.03	97.0	129.5	32.5	0.580	1.6	1.5	2.7
IV	A	3696	MS	M	99.69	88.5	116.0	27.5	0.491	1.8	1.8	3.6
	B	3697	H	M	98.61	88.0	121.5	33.5	0.598	1.8	1.8	2.9
	C	3688	MS	M	88.97	80.0	122.5	42.5	0.759	1.6	1.7	2.1
Average												2.8
V	A	3640[2]	BS	M	25.43	82.0	64.5	-17.5	-0.500	0.7	0.9	No Gain
	B	3786[3]	BS	M	34.53	98.0	80.0	-18.0	-0.429	0.8	0.9	No Gain
		3786	BS	M	23.53	80.0	96.0	16.0	1.143	1.7	1.9	1.5
	C	3784	H	M	55.06	77.0	92.0	15.0	0.268	1.0	1.2	3.7

1 Based on average weight of calves.
2 Removed at five weeks to save life.
3 Changed to adequate ration to save life at six weeks.

weight recommended by the National Research Council (89).

Calves in Group I required as an average 4.8 pounds of TDN for each pound of gain if calf 3603 which made no gain is not considered. Group II required only 2.9 pounds of TDN per pound of gain. Calves in Group III which received hydrogenated soybean oil, were relatively inefficient as they required 4.0 pounds of TDN per pound of gain. Calves in Group IV, on the higher plane of nutrition, required only 2.8 pounds of TDN per pound of gain. Calf 3688, in Group IV, which received butter oil made the most efficient gain, requiring only 2.1 pounds of TDN per pound of gain.

The lipid and milk intakes for the 8 to 12 week period are summarized in Table 28. The lactose, casein, fat and total TDN consumption during this period are listed for each calf for this same period. Table 29 shows the average daily gain, average TDN consumed per day, the average TDN consumed per 100 pounds of body weight, and the TDN consumed per pound of gain. The three calves receiving a lipid-free ration during this period consumed an average of 1.2 pounds of TDN daily per 100 pounds of body weight. Other calves in Group I received nutrients at a slightly higher level (averaging 1.3 pounds of TDN per 100 pounds of body weight). The one calf which remained in Group II received only 1.1

Table 28 Lipid and Milk Intake During Period from Eight to Twelve Weeks

Dietary Group	Sub-group	Calf No.	Breed	Sex	Weight at 8 Weeks	Age in Days 63	70	77	84	Total Milk Consumed	TDN from CHO	Prot.	Fat	Total TDN[1]
									(Lbs.)					
I		3596	H	F	72.5	70.0	70.0	58.0	58.0	256.0	12.54	8.42	11.18	32.14
		3597	H	F	85.0	68.6	68.6	98.5	98.0	333.7	16.36	10.98	-	27.34
		3603	H	M	95.0	81.2	72.1	71.4	71.4	296.1	14.51	9.74	9.68	33.93
		3768	MS	M	84.5	90.7	93.8	71.6	89.5	345.6	16.93	11.37	1.01	29.31
		3773	H	M	92.0	103.6	107.7	111.8	113.8	436.9	21.41	14.37	.67	36.45
		3780	H	M	78.0	88.3	95.9	98.0	100.8	383.0	18.77	12.61	2.02	33.40
		3699	H	M	92.5	102.1	106.4	107.0	106.0	421.5	20.66	13.87	-	34.53
		3700	H	M	103.0	88.3	113.9	120.4	122.7	445.3	21.82	14.65	-	36.47
Average					87.8	86.6	91.1	92.1	95.0	364.8	17.88	12.00	4.91[2]	32.95
II		3671	MS	M	124.5	97.0	98.0	105.0	109.5	409.5	20.07	13.47	17.87	51.41
III	A	3686	H	M	128.0	99.6	100.8	104.4	110.6	415.4	20.35	13.67	27.20	61.22
		3698	H	M	151.5	115.2	120.4	120.4	114.0	470.0	23.03	15.46	30.78	69.27
Average					139.8	107.4	110.6	112.4	112.3	442.7	21.69	14.57	28.99	65.25
	B	3701	H	M	129.5	100.8	99.4	106.4	112.0	418.6	20.51	13.77	27.40	61.68
IV	A	3696	MS	M	116.0	104.8	109.8	111.3	119.0	444.9	21.81	14.64	29.14	65.59
	B	3697	H	M	121.5	112.5	110.9	120.7	128.8	472.9	23.18	15.56	30.96	69.70
	C	3688	MS	M	122.5	106.2	114.6	120.5	128.1	469.4	23.00	15.44	30.74	69.18
V	B	3786[3]	BS	M	96.0	106.0				106.0	5.19	3.49	6.93	15.61
	C	3784	H	M	92.0	74.0	79.8	80.2	72.0	306.0	14.99	10.07	13.37	38.43

[1] Digestion coefficients used were, protein 94%, lactose 98% and fat 97% as given by Morrison (95).
[2] Average for animals receiving fat.
[3] Placed on adequate ration at six weeks in an attempt to obtain recovery.

Table 29 Effect of Dietary on Feed Intake of Calves during Period from Eight to Twelve Weeks

Dietary Group	Sub-group	Calf No.	Breed	Sex	TDN Consumed	Weight at 8 Weeks	Weight at 12 Weeks	Total Gain	Av. Daily Gain	Av. TDN Per Calf	Av. TDN Per 100/lbs[1] body weight	TDN per Lb. Gain
							(lbs)					
I		3597	H	F	27.34	85.0	85.5	0.5	0.018	1.0	1.1	54.7
		3699	H	M	34.53	92.5	104.0	11.5	0.410	1.2	1.3	3.0
		3700	H	M	36.47	103.0	116.0	13.0	0.464	1.3	1.2	2.8
Average					32.78	93.5	101.8	8.3	0.297	1.2	1.2	20.2
		3768	MS	M	29.31	84.5	84.0	-.5	No Gain	1.1	1.2	No Gain
		3773	H	M	36.45	92.0	101.1	9.0	0.321	1.3	1.3	4.1
		3780	H	M	33.40	78.0	100.0	22.0	0.786	1.2	1.4	1.5
		3596	H	F	32.14	72.5	74.0	1.5	0.054	1.2	1.5	21.4
		3603	H	M	33.93	95.0	100.5	5.5	0.196	1.2	1.3	6.2
Average					33.05	84.4	91.9	7.5	0.271	1.2	1.3	6.6
II		3671	MS	M	51.41	124.5	155.0	30.5	1.089	1.8	1.1	1.7
III	A	3686	H	M	61.22	128.0	151.0	23.0	0.821	2.2	1.3	2.7
		3698	H	M	69.27	151.5	165.5	14.0	0.500	2.5	1.0	5.0
Average					65.25	139.8	158.3	18.5	0.661	2.3	1.2	3.8
	B	3701	H	M	61.68	129.5	154.5	25.0	0.893	2.2	1.3	2.5
IV	A	3696	MS	M	65.59	116.0	138.0	22.0	0.786	2.3	1.7	3.0
	B	3697	H	M	69.70	121.5	150.0	28.5	1.018	2.5	1.6	2.5
	C	3688	MS	M	69.18	122.5	154.0	31.5	1.125	2.5	1.6	2.2
V	B	3786[2]	BS	M	15.61	96.0	106.0	10.0	1.429	2.2	2.2	1.6
	C	3784	H	M	38.43	92.0	114.0	22.0	0.786	1.4	1.3	1.8

[1] Per 100 lbs. weight, based on average weight of calves.
[2] Placed on adequate ration from 6 to 9 weeks, terminated at close of ninth week.

pounds of TDN per 100 pounds of body weight while calves in Groups III and IV received 1.3 and 1.6 pounds, respectively, of TDN daily per 100 pounds of body weight. The lipid-free group (Group I) required an average of 20.2 pounds of TDN per pound of gain whereas calves receiving lipids in attempts to effect recovery from fat deficiency syndromes required 6.2 pounds and one calf remaining in Group II required only 1.7 pounds of TDN per pound of gain. Requirements of animals in Groups III and IV ranged from 2.2 to 5.0 pounds of TDN for each pound of gain.

Lipid and milk intake for calves following the 12 week period are summarized in Table 30. Table 31 shows average daily gain, average TDN consumed per 100 pounds of body weight and TDN consumed per pound of gain. Calf 3597 responded poorly in attempts to obtain recovery but 3699 and 3700 responded to fat therapy and recovered in appearance and in ability to gain weight; these calves consumed 3.3 and 2.4 pounds of TDN, respectively, per pound of gain in weight. Calf 3773 made fairly efficient gains, requiring 2.2 pounds of TDN per pound of gain while receiving methyl linoleate as the only lipid. The most efficient gains, however, were made by 3768 which required only 1.6 pounds of TDN per pound of gain. This calf had responded poorly to lecithin but it responded very well when fat also was added to the ration.

Table 30 Lipid and Milk Intake for Calves Beyond the Twelve Week Period

Dietary Group	Calf No.	Breed	Sex	Weight 12 Weeks	Age in Days 91	98	105	112	119	126	133	140	147	Total Milk Consumed	TDN from CHO	Prot.	Fat	Total TDN[1]
					(Lbs.)									(Lbs.)	(Lbs.)	(Lbs.)	(Lbs.)	(Lbs.)
I	3597	H	F	85.5	83.0	83.0	83.0	83.0						332.0	16.27	10.92	21.74	48.93
	3699	H	M	104.0	81.7	82.9	93.2	84.7	86.9	89.8	86.3	94.0	97.3	796.8	39.04	26.22	34.79	100.05
	3700	H	M	116.0	91.7	93.9	93.4	95.5	99.0	104.6	110.5	116.7	122.2	927.5	45.44	30.51	40.48	116.43
	3773	H	M	101.0	115.4	117.6								233.0	11.42	7.67	1.01	20.10
	3768	MS	M	84.0	79.8	88.6	92.3							260.7	12.78	8.57	17.08	38.43

[1] Digestion coefficients used were, protein 94%, lactose 98% and fat 97% as given by Morrison (95).

Table 31 Effect of Dietary on Feed Efficiency for Calves Beyond the Twelve Week Period

Dietary Group	Calf No.	Breed	Sex	TDN Consumed	Initial Weight (Lbs.)	Final Weight (Lbs.)	Total Gain (Lbs.)	Average Daily Gain (Lbs.)	Av. Per Calf	TDN Per 100/lbs.[1] body weight	TDN per Lb. Gain
I	3597	H	F	48.93	85.5	85.0	-.5	No Gain	1.8	2.1	No Gain
	3699	H	M	100.05	104.0	134.5	30.5	.484	1.6	1.4	3.3
	3700	H	M	116.43	116.0	165.5	49.5	.786	1.9	1.2	2.4
	3773	H	M	20.10	101.0	110.0	9.0	.643	1.4	1.4	2.2
	3768	MS	M	38.43	84.0	108.0	24.0	1.143	1.8	1.9	1.6

[1] Per 100 lbs. weight, based on average weight of calves.

D. Plasma Fat Values

Blood plasma fat levels (Table 32) decreased during the first week in all of the calves which received colostrum regardless of their lipid intake. However, blood fat values in all calves started on the experimental ration without colostrum increased slightly (calves 3603, 3643, 3640 and 3688). Subsequent trends in the lipid-free group (Group I) were downward until a mean low value of 21 mg. per cent was reached at the seventh week. The mean blood plasma fat values during the period from 21 to 56 days were 24 and 163 mg. per 100 ml. for calves in Groups I and II, respectively. In groups where fat was fed the blood fat values began to increase the second week and increased until the fifth or sixth week at which time they tended to level off.

The blood fat levels rose very rapidly when fat or lipid fractions were introduced into the rations of calves previously fed only the semi-synthetic milk. Calves receiving crude soybean oil exhibited extremely high blood fat values. Blood fat values were higher in most instances when hydrogenated soybean oil and lecithin were fed than when at the same level hydrogenated soybean oil was fed. It was further observed that small amounts (0.2 per cent) of lipid fractions such as lecithin, free fatty acids,

Table 32 Effect of Dietary on the Blood Plasma "Allen Fat" Values

Dietary Group	Sub-group	Calf No.	Breed	Sex	Ration Syn. Milk[1]	Ration Lipid	Age in Days 0	7	14	21	28	35 (mg./100 ml.)	42	49	56	63	
I		3596	H	F	16	None	186.	64.	35.	33.	23.	14.	23.	36.	48.		
					11	2% H.S.B.O.										110.	1.
					11	2% B.O.											
		3597	H	F	16	None	192.	57.	43.	13.	12.	21.	17.	11.	44.	45.	[illegible]
					11	2% B.O.											
					11	2% B.O. + 2.0 Lec.											
		3603	H	M	16	None	21.	29.	30.	39.	43.	37.	46.	37.	38.		
					13	1.5% C.S.B.O.										152.	21
					11	1.5% C.S.B.O. + .5% Lec.											
		3768	MS	M	16	None	54.	53.	47.	24.	34.	28.	14.	10.	21.		
					16	7.5 g. Lec.										40.	[illegible]
					13	2.8% H.S.B.O. + .2% Lec.											
		3780	H	M	16	None	61.	41.	11.	21.	10.	13.	22.	11.	29.		
					16	Pork Liver = 15 g. Fat										57.	7
		3773	H	M	16	None	101.	24.	17.	20.	20.	13.	28.	9.	9.		
					16	5 g. Methyl Esters										22.	5
					16	15 g. Methyl Esters											
		3699	H	M	16	None	90.	76.	63.	33.	12.	24.	7.	15.	14.	19.	2
					11	2% C.S.B.O.											
					11	1.8% C.S.B.O. + .2% Lec.											
					11	1.8% H.S.B.O. + .2% Lec.											
		3700	H	M	16	None	75.	58.	75.	27.	33.	33.	14.	36.	39.	44.	1
					11	2% H.S.B.O.											
					11	1.8% H.S.B.O. + .2% Lec.											
Average 0-8 Weeks on Fat-free Ration							98.	50.	40.	26.	23.	23.	21.	21.	30.		
Average 8-12 Weeks on Fat-free Ration																36.	2
II		3657	BS	M	11	1.8% H.S.B.O. + .2% Lec.	115.	108.	155.	182.	205.	217.	204.	217.	248.		
		3643	BS	M	11	1.8% H.S.B.O. + .2% Lec.	21.	23.	61.	111.	123.	113.	145.	148.	100.		
		3671	MS	M	11	1.8% H.S.B.O. + .2% Lec.	120.	110.	105.	117.	113.	149.	203.	106.	162.	163.	16
Average							85.	80.	107.	137.	147.	160.	184.	177.	170.		
III	A	3686	H	M	11	3% H.S.B.O.	154.	36.	27.	59.	107.	118.	109.	126.	123.	125.	13
		3698	H	M	11	3% H.S.B.O.	150.	67.	93.	62.	61.	87.	113.	140.	161.	176.	7
Average							152.	52.	60.	61.	84.	103.	111.	133.	142.	151.	10
	B	3701	H	M	11	2.8% H.S.B.O. + .2% Lec.	134.	15.	103.	131.	145.	221.	82.	224.	262.	314.	22
IV	A	3696	MS	M	13	3% H.S.B.O.	140.	65.	55.	68.	95.	63.	67.	139.	114.	101.	9
	B	3697	H	M	13	2.8% H.S.B.O. + .2% Lec.	70.	65.	68.	90.	122.	112.	74.	137.	115.	128.	13
	C	3688[2]	MS	M	13	3% B.O.	25.	28.	36.	128.	171.	183.	215.	245.	257.	300.	27
Average							78.	52.	53.	95.	129.	119.	119.	174.	165.	176.	16
V	A	3640	BS	M	16	5 g. Lec.	21.	45.	26.	84.	92.	99.					
	B	3786	BS	M	16	5 g. F.F.A.	65.	29.	15.	17.	60.	42.					
					16	15 g. F.F.A.							58.				
					13	2.8% H.S.B.O. + .2% Lec.								109.	98.	123.	
	C	3784	H	M	16	5 g. F.F.A.	38.	30.	18.	50.	76.	105.					
					16	15 g. F.F.A.							135.	136.	210.		
					11	1.8% H.S.B.O. + .2% Lec.										234.	25

[1] Per cent of body weight daily.
[2] On fat-free ration first two weeks, at rate of 16 per cent of body weight.

.etary on the Blood Plasma "Allen Fat" Values

Age in Days																						
0	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140	147
					(mg./100 ml.)																
.86.	64.	36.	33.	23.	14.	23.	36.	48.	110.	123.	96.	95.	171.								
92.	57.	43.	13.	12.	21.	17.	11.	44.	45.	24.	43.	42.	77.	188.	409.	467.					
21.	29.	30.	39.	43.	37.	46.	37.	38.	152.	245.	329.	405.	385.	379.	385.	352.					
54.	53.	47.	24.	34.	28.	14.	10.	21.	40.	38.	53.	41.	130.	172.	169.						
61.	41.	11.	21.	10.	13.	22.	11.	29.	57.	72.	117.	116.									
01.	24.	17.	20.	20.	13.	28.	9.	9.	22.	51.	47.	23.	55.	71.							
90.	76.	63.	33.	12.	24.	7.	15.	14.	19.	26.	25.	33.	80.	156.	177.	185.	181.	269.	229.	257.	306.
75.	58.	75.	27.	33.	33.	14.	36.	39.	44.	15.	36.	50.	91.	49.	161.	137.	140.	200.	223.	220.	249.
98.	50.	40.	26.	23.	23.	21.	21.	30.	36.	22.	35.	42.									
15.	108.	155.	182.	205.	217.	204.	237.	248.													
21.	23.	61.	111.	123.	113.	145.	148.	100.													
20.	110.	105.	117.	113.	149.	203.	106.	162.	163.	167.	171.	211.									
85.	80.	107.	137.	147.	160.	184.	177.	170.													
54.	36.	27.	59.	107.	118.	109.	126.	123.	125.	137.	186.	204.									
50.	67.	93.	62.	61.	87.	113.	140.	161.	176.	78.	84.	111.									
52.	52.	60.	61.	84.	103.	111.	133.	142.	151.	108.	135.	158.									
34.	15.	103.	131.	145.	221.	82.	224.	262.	314.	223.	170.	184.									
40.	65.	55.	68.	95.	63.	67.	139.	114.	101.	91.	100.	82.									
70.	65.	68.	90.	122.	112.	74.	137.	115.	128.	130.	168.	160.									
25.	28.	36.	128.	171.	183.	215.	245.	267.	300.	276.	226.	186.									
78.	52.	53.	95.	129.	119.	119.	174.	165.	176.	166.	165.	143.									
21.	45.	26.	84.	92.	99.																
55.	29.	15.	17.	60.	42.	58.	109.	98.	123.												
38.	30.	18.	50.	76.	105.	135.	136.	210.	234.	254.	261.	255.									

reight.

methyl esters and pork liver fat tended to increase the "Allen fat" value considerably over that of calves in the lipid-free group.

E. Plasma Phospholipid Values

Data relating to blood phospholipids are shown in Table 33. The method developed by Zilversmit and Davis (141) was employed in determining the phospholipids. The phospholipid content of the trichloroacetic acid precipitate was estimated by simply multiplying phosphorus content by 25. It was realized that this method may possibly measure some non-lipid phosphorus in addition to the phospholipid phosphorus. If this is the case, it is likely that all values were too high but since the errors in all cases would be in the same direction, comparison still might be valid.

The mean phospholipid values of calves in Groups I and II during the 21 to 56 day period were 54.4 and 138.5 mg. per 100 ml. of plasma, respectively. Calves which received hydrogenated soybean oil plus lecithin had slightly higher plasma phospholipid values than calves receiving only hydrogenated soybean oil. These data suggest that the phospholipid content of plasma is enhanced by inclusion of lipid in the dietary of the calf.

Table 33 Effect of Dietary on the Blood Plasma Phospholipid Values

Dietary Group	Sub-group	Calf No.	Breed	Sex	Ration: Syn. Milk[1]	Ration: Lipid	Age in Days (mg./100 ml.): 0	7	14	21	28	35	42	49	56	63	70	77	8[illegible]
I		3768	MS	L	16	None	80.3	69.5	56.8	39.0	79.0	53.3	65.0	30.0	53.5				
					16	7.5 g. Lec.										59.5	64.8	70.3	80.
					13	2.8% H.S.B.O. + .2% Lec.													
		3780	H	M	16	None	78.3	90.0	43.0	46.8	42.5	49.8	51.8	70.0	50.5				
					16	Pork Liver ≈ 15 g. Fat										69.5	101.3	117.5	116.
		3773	H	M	16	None	114.5	96.8	48.8	65.0	29.3	55.5	63.3	49.5	52.0				
					16	5 g. Methyl Esters										76.8	78.0	75.8	99.
					16	15 g. Methyl Esters													
		3699	H	—	16	None	125.5	95.0	42.3	56.5	40.0	64.0	43.8	76.5	37.0	42.0	50.8	45.5	31.
					11	2% C.S.B.O.													
					11	1.8% C.S.B.O. + .2% Lec.													
					11	1.8% H.S.B.O. + .2% Lec.													
		3700	H	M	16	None	83.5	75.0	56.0	59.5	52.5	73.5	51.5	74.0	56.0	67.8	56.0	57.0	36.
					11	2% H.S.B.O.													
					11	1.8% H.S.B.O. + .2% Lec.													
Average 0-8 Weeks on Fat-free Ration							96.4	85.3	49.4	53.4	48.7	59.2	55.1	60.0	49.8				
Average 8-12 Weeks on Fat-free Ration																54.9	53.4	51.3	34.
II		3657	BS	M	11	1.8% H.S.B.O. + .2% Lec.	17.3	104.5	83.0	163.5	153.0	191.7	196.0	175.0	166.9				
		3643	BS	M	11	1.8% H.S.B.O. + .2% Lec.	46.2	43.4	60.4	98.6	118.5	99.3	76.0	105.5	111.0				
		3671	MS	M	11	1.8% H.S.B.O. + .2% Lec.	125.0	120.8	104.5	139.0	120.3	142.5	152.6	144.5	138.3	176.0	163.5	175.0	159.
Average							62.8	89.6	82.6	133.7	130.6	144.5	141.5	141.7	138.7				
III	A	3686	H	M	11	3% H.S.B.O.	136.5	73.8	68.0	75.9	124.3	108.8	147.0	150.5	119.5	140.5	149.0	157.0	219.
		3698	H	M	11	3% H.S.B.O.	158.5	92.5	77.0	102.5	93.5	109.0	120.0	140.5	156.0	169.0	119.5	105.0	110.
Average							147.0	83.1	72.5	89.2	108.9	108.9	133.5	145.5	137.8	154.8	134.3	131.0	164.
	B	3701	H	M	11	2.8% H.S.B.O. + .2% Lec.	119.0	48.0	96.5	112.5	146.0	186.0	106.0	182.0	243.0	233.8	209.8	153.0	226.
IV	A	3696	MS	M	13	3% H.S.B.O.	135.0	92.5	97.5	88.0	73.0	67.0	70.0	132.0	114.5	94.8	116.8	120.5	104.
	B	3697[2]	H	M	13	2.8% H.S.B.O. + .2% Lec.	110.0	101.3	79.0	99.0	92.5	111.0	111.0	178.0	111.0	112.0	156.0	159.0	160.
	C	3688[2]	MS	M	13	3% B.O.	57.0	58.3	60.0	90.0	121.5	135.0	150.5	188.5	153.5	202.0	214.0	178.0	187.
Average							100.7	84.0	78.8	92.3	95.7	104.3	110.5	166.2	126.3	136.3	162.3	152.5	150.
V	B	3786	BS	M	16	5 g. F.F.A.	76.5	52.8	48.0	44.5	58.3	69.8							
					16	15 g. F.F.A.							87.8						
					13	2.8% H.S.B.O. + .2% Lec.								92.5	133.5	175.8			
	C	3784	H	M	16	5 g. F.F.A.	77.8	52.8	48.0	68.0	78.5	101.5							
					16	15 g. F.F.A.							141.0	113.0	131.3				
					11	1.8% H.S.B.O. + .2% Lec.										243.0	208.3	235.3	240.

[1] Per cent of body weight daily.
[2] On fat-free ration first two weeks.

ctary on the Blood Plasma Phospholipid Values

Age in Days																					
0	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140	147
					(mg./100 ml.)																
80.3	69.5	56.8	39.0	79.0	53.3	65.0	30.0	53.5													
									59.5	64.8	70.3	80.5									
													98.5	177.5	213.8						
78.3	90.0	43.0	46.8	42.5	49.8	51.8	70.0	50.5													
									69.5	101.3	117.5	116.8									
114.5	96.8	48.8	65.0	29.3	55.5	63.3	49.5	52.0													
									76.8	78.0	75.8	99.0									
													120.0	111.3							
125.5	95.0	42.3	56.5	40.0	64.0	43.8	76.5	37.0	42.0	50.8	45.5	31.8									
													94.5	158.8	117.5	126.8	100.8				
																		176.8	146.0		
																				135.0	126.9
83.5	75.0	56.0	59.5	52.5	73.5	51.5	74.0	56.0	67.8	56.0	57.0	36.5									
													77.0	127.5	110.5	93.3	80.3				
																		166.0	122.0	141.0	190.0
96.4	85.3	49.4	53.4	48.7	59.2	55.1	60.0	49.8													
									54.9	53.4	51.3	34.1									
17.3	104.5	83.0	163.5	153.0	191.7	196.0	175.0	166.9													
46.2	43.4	60.4	98.6	118.5	99.3	76.0	105.5	111.0													
125.0	120.8	104.5	139.0	120.3	142.5	152.6	144.5	138.3	176.0	163.5	175.0	159.0									
62.8	89.6	82.6	133.7	130.6	144.5	141.5	141.7	138.7													
136.5	73.8	68.0	75.9	124.3	108.8	147.0	150.5	119.5	140.5	149.0	157.0	219.0									
158.5	92.5	77.0	102.5	93.5	109.0	120.0	140.5	156.0	169.0	119.5	105.0	110.0									
147.0	83.1	72.5	89.2	108.9	108.9	133.5	145.5	137.8	154.8	134.3	131.0	164.5									
119.0	48.0	96.5	112.5	146.0	186.0	106.0	182.0	243.0	233.8	209.8	153.0	226.0									
135.0	92.5	97.5	88.0	73.0	67.0	70.0	132.0	114.5	94.8	116.8	120.5	104.3									
110.0	101.3	79.0	99.0	92.5	111.0	111.0	178.0	111.0	112.0	156.0	159.0	160.0									
57.0	58.3	60.0	90.0	121.5	135.0	150.5	188.5	153.5	202.0	214.0	178.0	187.0									
100.7	84.0	78.8	92.3	95.7	104.3	110.5	166.2	126.3	136.3	162.3	152.5	150.4									
76.5	52.8	48.0	44.5	58.3	69.8																
						87.8															
							92.5	133.5	175.8												
77.8	52.8	48.0	68.0	78.5	101.5																
						141.0	113.0	131.3													
									243.0	208.3	235.3	240.5									

The effect of diet on changes in group mean plasma phospholipid values of the various groups are summarized in Figure 4.

F. Plasma Total Fatty Acid Values

Blood plasma total fatty acid values (Table 34) decreased during the first week in all calves on the lipid-free, semi-synthetic milk (Group I). The lowest mean total fatty acid value in the lipid-free group was 18 mg. per cent at seven weeks of age. The mean total fatty acid values during the period from 21 to 56 days were 22 and 143 mg. per 100 ml. for calves on the lipid-free milk (Group I) and semi-synthetic milk containing lipids (Group II), respectively.

Figure 5 shows the effect of dietary on changes in group mean blood plasma "Allen fat" and total fatty acid in blood plasma. The similarity of group means in the two diagrams is noteworthy.

G. Plasma Polyunsaturated Fatty Acid Values

The linoleic acid values in the blood plasma (Table 35) declined sharply after the calves had received the lipid-free

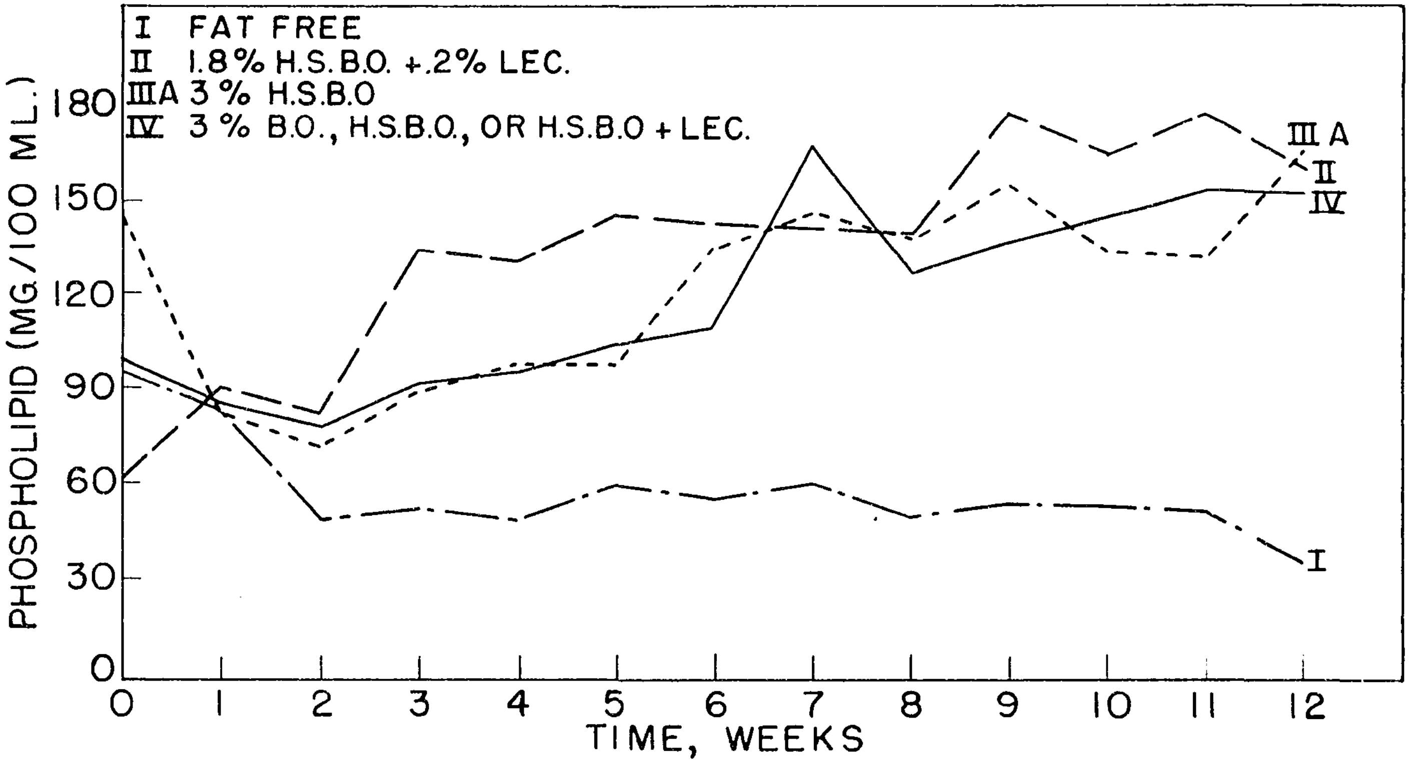

Figure 4. Effect of dietary on changes in group mean plasma phospholipid values

Table 34 Effect of Dietary on the Plasma Total Fatty Acid Values

Dietary Group	Sub-group	Calf No.	Breed	Sex	Ration: Syn. Milk[1]	Ration: Lipid	Age in Days: 0	7	14	21	28	35	42	49	56	63	7
									(mg./100 ml.)								
I		3596	H	F	16	None	143.2	55.5	40.0	34.0	29.0	41.0	26.2	32.8	36.4		
					11	2% H.S.B.O.										85.6	88.
					11	2% B.O.											
		3597	H	F	16	None	132.5	52.4	32.0	18.2	14.0	27.7	9.3	25.0	44.3	35.0	46.
					11	2% B.O.											
					11	2% B.O. + 2% Lec.											
		3603	H	M	16	None	50.6	38.3	31.1	36.6	17.7	38.3	24.3	38.4	37.2		
					13	1.5% C.S.B.O.										121.4	95.
					11	1.5% C.S.B.O. + .5% Lec.											
		3768	MS	M	16	None	34.4	24.8	28.0	10.0	32.0	29.1	38.0	7.0	4.2		
					16	7.5 g. Lec.										37.4	44.
					13	2.8% H.S.B.O. + .2% Lec.											
		3780	H	M	16	None	108.7	42.9	17.5	12.2	11.9	10.8	17.8	10.5	7.5		
					16	Pork Liver ⇐ 15 g. Fat										11.1	26.
		3773	H	M	16	None	96.3	22.8	16.0	19.8	24.9	8.4	29.5	7.2	10.4		
					16	5 g. Methyl Esters										3.5	9.
					16	15 g. Methyl Esters											
		3699	H	M	16	None	82.5	61.8	32.0	16.9	10.8	12.9	27.7	11.3	10.9	26.7	25.
					11	2% C.S.B.O.											
					11	1.8% C.S.B.O. + .2% Lec.											
					11	1.8% H.S.B.O. + 2% Lec.											
		3700	H	M	16	None	62.5	43.0	59.5	10.4	16.1	32.4	50.3	13.4	27.2	41.3	44.
					11	2% H.S.B.O.											
					11	1.8% H.S.B.O. + .2% Lec.											
Average 0-8 Weeks on Fat-free Ration							86.3	42.7	32.0	19.8	19.6	25.1	27.9	18.2	22.3		
Average 8-12 Weeks on Fat-free Ration																34.3	38.
II		3657	BS	M	11	1.8% H.S.B.O. + .2% Lec.	77.4	111.6	124.1	135.7	176.6	207.9	180.4	191.5	232.4		
		3643	BS	M	11	1.8% H.S.B.O. + .2% Lec.	15.0	14.0	36.8	87.2	98.8	88.0	92.4	124.6	119.9		
Average							46.2	62.8	80.5	111.5	137.7	147.9	136.4	148.0	176.2		
III	A	3685	H	M	11	3% H.S.B.O.	147.3	44.8	52.3	69.7	113.4	66.6	128.6	134.4	130.0		
V	A	3640	BS	M	16	5 g. Lec.	6.1	62.7	28.0	49.3	52.3	50.0					
	B	3786	BS	M	16	5 g. F.F.A.	52.3	33.2	4.8	4.7	15.8	26.9					
					16	15 g. F.F.A.							41.3				
					13	2.8% H.S.B.O. + .2% Lec.								46.0	73.3	45.3	
	C	3784	H	M	16	15 g. F.F.A.	27.0	19.3	8.2	31.3	29.5	101.7					
					16	15 g. F.F.A.							47.3	48.1	85.3		
					11	1.8% H.S.B.O. + .2% Lec.										50.8	196.

[1] Per cent of body weight daily

n the Plasma Total Fatty Acid Values

Age in Days																				
7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140	147
	(mg./100 ml.)																			
55.5	40.0	34.0	29.0	41.0	26.2	32.8	36.4													
								85.6	88.5	58.1										
											88.1	121.4								
52.4	32.0	18.2	14.0	27.7	9.3	25.0	44.3	35.0	46.0	29.0	40.0									
												73.6	163.1							
														295.6						
38.3	31.1	36.6	17.7	38.3	24.3	38.4	37.2													
								121.4	95.7	185.1	256.1	290.8								
													277.3	194.4						
24.8	28.0	10.0	32.0	29.1	38.0	7.0	4.2													
								37.4	44.0	48.5	84.2									
												35.7	37.7	40.3						
42.9	17.5	12.2	11.9	10.8	17.8	10.5	7.5													
								11.1	26.0	52.0	49.1									
22.8	16.0	19.8	24.9	8.4	29.5	7.2	10.4													
								3.5	9.0	10.8	9.1									
												20.7	14.0							
61.8	32.0	16.9	10.8	12.9	27.7	11.3	10.9	26.7	25.2	18.6	14.2									
												78.0	112.4	196.9	231.7	300.0				
																	227.1	245.0		
																			271.3	280.0
43.0	59.5	10.4	16.1	32.4	50.3	13.4	27.2	41.3	44.3	12.5	31.4									
												86.4	107.9	183.7	157.4	177.1				
																	108.8	180.0	179.5	194.7
42.7	32.0	19.8	19.6	25.1	27.9	18.2	22.3													
								34.3	38.5	20.1	28.5									
111.6	124.1	135.7	176.6	207.9	180.4	191.5	232.4													
14.0	36.8	87.2	98.8	88.0	92.4	124.6	119.9													
62.8	80.5	111.5	137.7	147.9	136.4	148.0	176.2													
44.8	52.3	69.7	113.4	66.6	128.6	134.4	130.0													
62.7	28.0	49.3	52.3	50.0																
33.2	4.8	4.7	15.8	26.9																
					41.3															
						46.0	73.3	45.3												
19.3	8.2	31.3	29.5	101.7																
					47.3	48.1	85.3													
								50.8	196.3	143.7	182.2									

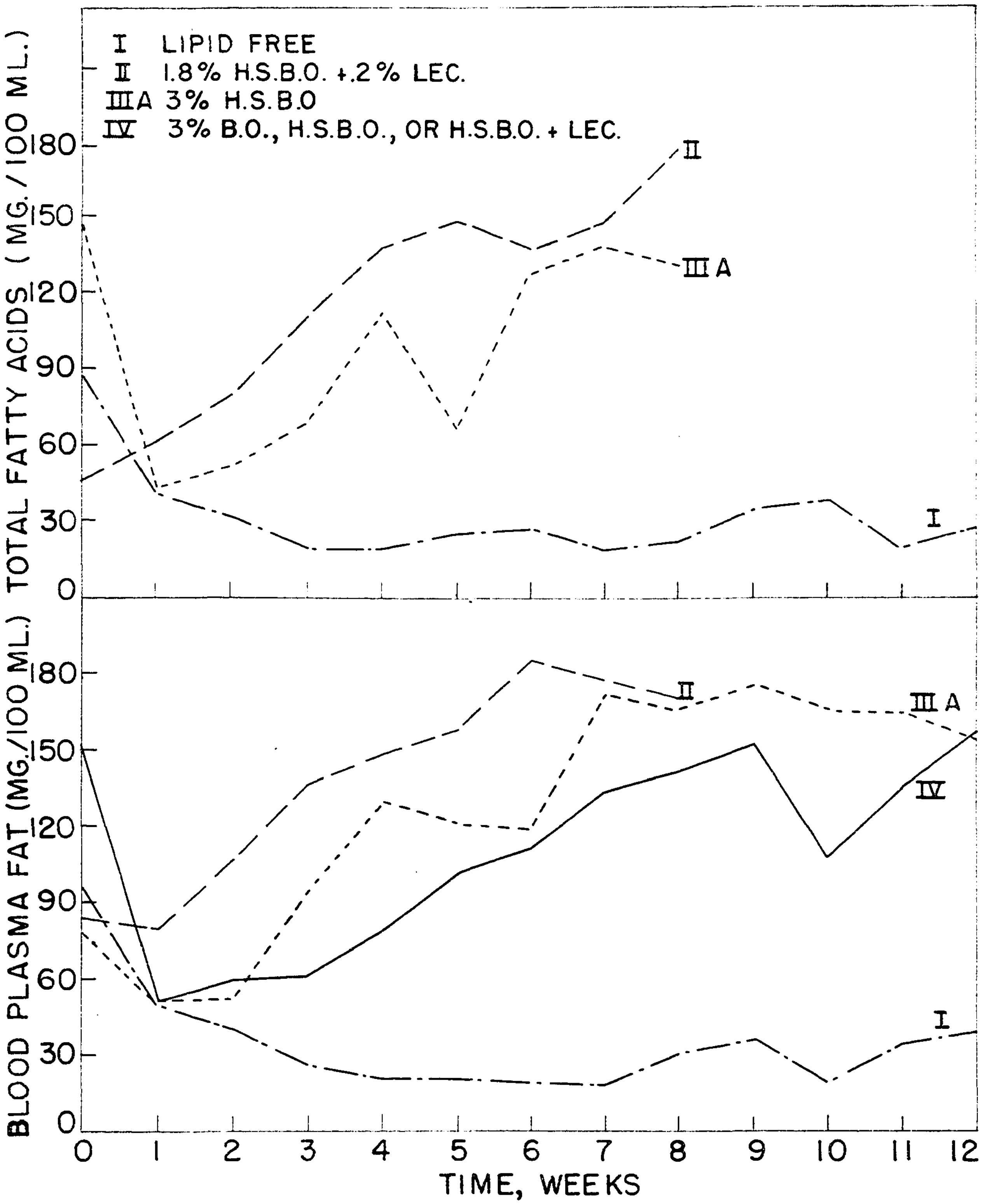

Figure 5. Effect of dietary on changes in group mean "Allen fat" and total fatty acid blood plasma

Table 35 Effect of Dietary on Blood Plasma I

Dietary Group	Sub-group	Calf No.	Breed	Sex	Ration: Syn. Milk[1]	Ration: Lipid	0	7	14	21	28	35	42	49	56
													(mg./100 ml.)		
I		3596	H	F	16	None	26.4	6.4	3.9	2.7	2.0	4.0	3.9	3.3	3.1
					11	2% H.S.B.O.									
					11	2% B.O.									
		3597	H	F	16	None	32.7	7.8	1.8	3.4	1.6	2.4	1.7	2.3	4.9
					11	2% B.O.									
					11	2% B.O. + 2% Lec.									
		3603	H	M	16	None	14.0	4.3	1.4	2.3	2.6	3.6	2.8	3.8	3.0
					13	1.5% C.S.B.O.									
					11	1.5% C.S.B.O. + .5% Lec.									
		3768	MS	M	16	None	2.4	-	-	-	-	1.0	1.0	1.0	0.3
					16	7.5 g. Lec.									
					13	2.8% H.S.B.O. + .2% Lec.									
		3780	H	M	16	None	6.9	1.6	2.5	1.0	0.9	0.4	0.3	1.1	0.0
					16	Pork Liver ⇌ 15 g. Fat									
		3773	H	M	16	None	5.2	3.5	0.0	0.6	0.9	0.1	2.4	.7	0.0
					16	5 g. Methyl Esters									
					16	15 g. Methyl Esters									
		3699	H	M	16	None	-	-	-	1.7	1.0	3.2	1.6	0.4	0.3
					11	2% C.S.B.O.									
					11	1.8% C.S.B.O. + .2% Lec.									
		3700	H	M	16	None	-	-	-	2.4	4.6	11.7	4.4	1.5	1.6
					11	2% H.S.B.O.									
					11	1.8% H.S.B.O. + .2% Lec.									
Average 0-8 Weeks on Fat-free Ration							14.6	4.7	1.9	2.0	2.0	3.3	2.9	1.7	1.7
Average 8-12 Weeks on Fat-free Ration															
II		3657	BS	M	11	1.8% H.S.B.O. + .2% Lec.	22.1	47.6	72.6	85.0	54.5	129.2	112.0	95.7	129.0
		3643	BS	M	11	1.8% H.S.B.O. + .2% Lec.	21.9	21.6	34.1	39.8	60.2	56.2	58.7	50.1	36.9
Average							22.0	34.6	53.4	62.4	57.3	93.0	85.3	72.9	83.0
III	A	3686	H	M	11	3% H.S.B.O.	35.4	11.4	8.4	11.0	31.9	26.9	55.8	31.7	32.0
V	A	3640	BS	M	16	5 g. Lec.	1.7	19.7	11.2	21.2	21.0	19.2			
	B	3736	BS	M	16	5 g. F.F.A.	3.7	0.2	0.5	2.3	4.9	18.5			
					16	15 g. F.F.A.							23.0	32.8	13.4
	C	3784	H	M	16	5 g. F.F.A.	8.0	6.3	6.4	6.7	6.7	15.4	21.4	20.2	33.6
					16	15 g. F.F.A.									
					11	1.8% H.S.B.O. + .2% Lec.									

[1] Per cent of body weight daily

Table 35 Effect of Dietary on Blood Plasma Linoleic Acid Values

	0	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126
							(mg./100 ml.)												
	26.4	6.4	3.9	2.7	2.0	4.0	3.9	3.3	3.1										
										13.3	13.9	10.1							
													17.7	43.8					
	32.7	7.8	1.8	3.4	1.6	2.4	1.7	2.3	4.9	3.8	5.5	4.3	4.2						
														19.9	20.0				
																77.9			
	14.0	4.3	1.4	2.3	2.6	3.6	2.8	3.8	3.0										
										67.3	58.6	124.9	151.7	197.8					
Lec.															183.2	186.7			
	2.4	-	-	-	-	1.0	1.0	1.0	0.3										
										7.9	1.2	8.3	3.0						
Lec.														2.7	11.2	14.9			
	6.9	1.6	2.5	1.0	0.9	0.4	0.3	1.1	0.0										
Fat										1.3	5.4	5.3	2.6						
	5.2	3.5	0.0	0.6	0.9	0.1	2.4	.7	0.0										
										0.4	2.7	2.7	1.5						
														8.7	2.8				
	-	-	-	1.7	1.0	3.2	1.6	0.4	0.3	4.2	1.6	1.1	0.4						
														28.9	43.1	78.2	88.4	79.1	
Lec.																			130.8
	-	-	-	2.4	4.6	11.7	4.4	1.5	1.6	3.4	0.8	0.5	0.7						
														18.7	21.8	25.9	38.4	22.0	
Lec.																			22.3
	14.6	4.7	1.9	2.0	2.0	3.3	2.9	1.7	1.7										
										3.8	2.6	2.0	1.8						
Lec.	22.1	47.6	72.6	85.0	54.5	129.2	112.0	95.7	129.0										
Lec.	21.9	21.6	34.1	39.8	60.2	56.2	58.7	50.1	36.9										
	22.0	34.6	53.4	62.4	57.3	93.0	85.3	72.9	83.0										
	35.4	11.4	8.4	11.0	31.9	26.9	55.8	31.7	32.0										
	1.7	19.7	11.2	21.2	21.0	19.2													
	3.7	0.2	0.5	2.3	4.9	18.5													
							23.0	32.8	13.4										
	8.0	6.3	6.4	6.7	6.7	15.4	21.[illegible]	20.2	33.6										
										22.5	51.2	27.9							
Lec.													97.7						

milk for one week and remained consistently low throughout the experimental period. Linoleic acid values for calves in the lipid-free group during the period from 21 to 56 days was 2.3 mg. per cent, whereas calves receiving hydrogenated soybean oil and lecithin (Group II) averaged 75.7 mg. per cent of linoleic acid over the same period. Feeding of 15 grams of pork liver fat or 15 grams of methyl esters increased the linoleic acid values slightly. Group V calves which received small amounts of free fatty acids had variable but significantly higher plasma linoleic acid values than did calves in the lipid-free group.

The relationship of blood plasma linoleic acid values to body weight changes were evaluated statistically[1]. The correlation within calves where differences between calves have been taken out (Groups I and II) are summarized in Table 36. These data suggest that a small positive correlation existed between linoleic acid values and weight change in the case of the lipid-free calves. A somewhat higher positive correlation was found (Group II) when calves received lipids.

The arachidonic acid content (Table 37) in the blood plasma of the calves on the fat-free ration (Group I) de-

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[1]All statistical methods used in analyzing the data in this thesis were taken from Snedecor (126).

Table 36

Correlation of Blood Plasma Linoleic
Acid to Body Weight

Group	Calf No.	SX_2	SY_2	SXY
I	3596	11.87	40.10	8.71
	3597	32.31	59.50	1.70
	3603	5.94	33.00	5.75
	3768	0.37	0.78	0.16
	3780	3.26	30.88	1.58
	3773	11.40	36.50	-11.35
	3699	6.90	27.92	- 3.93
	3700	77.50	34.79	24.63
Total		149.55	263.47	27.25
		$r = 27.25/\sqrt{(149.55)\ (263.46)} = .14$		
II	3657	6963.70	70.40	173.00
	3643	682.20	113.69	260.36
Total		7645.90	184.09	433.36
		$r = 433.36/\sqrt{(7645.90)\ (184.09)} = .37$		

Table 37 Effect of Dietary on Blood Plasma Arac

Dietary Group	Sub-group	Calf No.	Breed	Sex	Ration: Syn. Milk[1]	Ration: Lipid	Age in Days: 0	7	14	21	28	35	42	49	56	63	7
													(mg./100 ml.)				
I		3596	H	F	16	None	11.0	8.6	7.4	4.0	2.1	0.7	3.8	4.8	2.9		
					11	2% H.S.B.O.										4.7	5.
					11	2% B.O.											
		3597	H	F	16	None	12.4	8.0	2.9	0.0	1.9	2.6	2.3	2.3	5.0	4.2	4.
					11	2% B.O.											
					11	2% B.O. + 2% Lec.											
		3603	H	M	16	None	3.5	2.0	2.3	2.8	2.6	3.8	1.9	4.3	3.2	4.3	3.
					13	1.5% C.S.B.O.											
					11	1.5% C.S.B.O. + .5% Lec.											
		3758	MS	M	16	None	2.0	-	-	-	-	2.3	0.5	1.0	0.0		
					16	7.5 g. Lec.										1.7	2.
					13	2.7% H.S.B.O. + .2% Lec.											
		3780	H	M	16	None	7.4	1.3	2.1	0.9	1.8	0.0	0.8	2.5	0.0		
					16	Pork Liver ⇔ 15 g. Fat										0.0	5.
		3773	H	M	16	None	2.6	1.5	0.0.	1.7	1.7	0.0	1.7	0.3	0.0		
					16	5 g. Methyl Esters										0.3	0.
					16	15 g. Methyl Esters											
		3699	H	M	16	None	-	-	-	1.1	0.0	0.5	1.4	0.0	0.0	1.5	1.
					11	2% C.S.B.O.											
					11	1.8% C.S.B.O. + 12% Lec.											
		3700	H	M	16	None	-	-	-	0.0	0.7	5.5	0.0	0.6	1.1	5.1	2.
					11	2% H.S.B.O.											
					11	1.8% H.S.B.O. + .2% Lec.											
Average 0-8 Weeks on Fat-free Ration							6.5	4.3	2.9	1.5	1.5	1.9	1.6	2.0	1.5		
Average 8-12 Weeks on Fat-free Ration																3.6	2.6
II		3657	BS	M	11	1.8% H.S.B.O. + .2% Lec.	6.3	10.4	6.3	7.8	3.5	4.4	3.5	2.6	8.0		
		3643	BS	M	11	1.8% H.S.B.O. + .2% Lec.	0.6	0.6	0.0	2.9	2.4	2.9	2.2	1.3	7.3		
Average							3.4	5.5	3.2	5.4	3.0	3.7	2.8	1.9	7.7		
III	A	3686	H	M	11	3% H.S.B.O.	7.7	6.6	5.2	5.6	7.8	5.5	8.8	4.5	4.0		
V	A	3640	BS	M	16	5 g. Lec.	0.2	4.3	2.0	3.3	2.4						
	B	3786	BS	M	16	5 g. F.F.A.	-	3.7	0.4	0.0	0.8						
					16	15 g. F.F.A.						1.3	2.6				
					13	2.8% H.S.B.O. +.2% Lec.								2.0	1.9	1.1	
	C	3784	H	M	16	5 g. F.F.A.	2.1	1.4	2.1	2.8	4.0	2.3					
					16	15 g. F.F.A.							3.2	2.6	4.9		
					11	1.8% H.S.B.O. + .2% Lec.										2.1	1.6

[1] Per cent of body weight daily

Table 37 Effect of Dietary on Blood Plasma Arachidonic Acid Values

	Age in Days																		
	0	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126
							(mg./100 ml.)												
	11.0	8.6	7.4	4.0	2.1	0.7	3.8	4.8	2.9										
										4.7	5.7	3.5							
													4.9	10.8					
	12.4	8.0	2.9	0.0	1.9	2.6	2.3	2.3	5.0	4.2	4.6	2.5	2.2						
														7.1	7.8				
:.																8.7			
	3.5	2.0	2.3	2.8	2.6	3.8	1.9	4.3	3.2	4.3	3.2								
												3.0	4.2	6.0	4.5				
.5% Lec.																4.5	4.3	1.0	
	2.0	-	-	-	-	2.3	0.5	1.0	0.0										
										1.7	2.2	1.0	0.0						
.2% Lec.														0.0	0.8	0.4	·	0.0	
	7.4	1.3	2.1	0.9	1.8	0.0	0.8	2.5	0.0										
5 g. Fat										0.0	5.7	5.5	3.3						
	2.6	1.5	0.0	1.7	1.7	0.0	1.7	0.3	0.0										
rs										0.3	0.7	0.0	0.0						
ers														2.2	0.1				
	-	-	-	1.1	0.0	0.5	1.4	0.0	0.0	1.5	1.1	0.4	0.4						
														1.7	0.0	3.3	1.3	1.1	
12% Lec.																			21.6
	-	-	-	0.0	0.7	5.5	0.0	0.6	1.1	5.1	2.1	0.6	1.3						
														4.8	3.3	3.7	4.1	4.6	
.2% Lec.																			1.8
	6.5	4.3	2.9	1.5	1.5	1.9	1.6	2.0	1.5										
										3.6	2.6	1.1	1.3						
.2% Lec.	6.3	10.4	6.3	7.8	3.5	4.4	3.5	2.6	8.0										
.2% Lec.	0.6	0.6	0.0	2.9	2.4	2.9	2.2	1.3	7.3										
	3.4	5.5	3.2	5.4	3.0	3.7	2.8	1.9	7.7										
	7.7	6.6	5.2	5.6	7.8	5.5	8.8	4.5	4.0										
	0.2	4.3	2.0	3.3	2.4														
	-	3.7	0.4	0.0	0.8														
						1.3	2.6												
2% Lec.								2.0	1.9	1.1									
	2.1	1.4	2.1	2.8	4.0	2.3													
							3.2	2.6	4.9										
.2% Lec.										2.1	1.6	2.9	4.9						

clined until about the third week at which time there was a trend toward plateauing. The mean blood plasma arachidonic acid values for calves during the period from 21 to 56 days were 1.3 and 4.1 mg. per cent for Groups I and II, respectively. No arachidonic acid could be found in four of the eight plasma samples of calves on the lipid-free ration (Group I) at eight weeks of age. Plasma arachidonic acid values increased sharply in most instances when lipids were added to the ration.

Table 38 shows the linolenic acid content in the blood plasma of the calves. Linolenic acid was very low in both lipid-free and lipid-supplemented calves as indicated by the mean values for the 21 to 56 day period which were 0.3 and 1.8 mg. per 100 ml. for Groups I and II, respectively. The amount found in each of those groups was variable; in many instances only traces of linolenic acid were present in plasma samples from individual calves.

Figure 6 summarizes the effect of diet on the average blood plasma changes of linoleic, linolenic and arachidonic fatty acids. The amounts of linolenic and arachidonic acids in the blood plasma were relatively small in both the lipid-free and the supplemented groups as compared with the linoleic acid values.

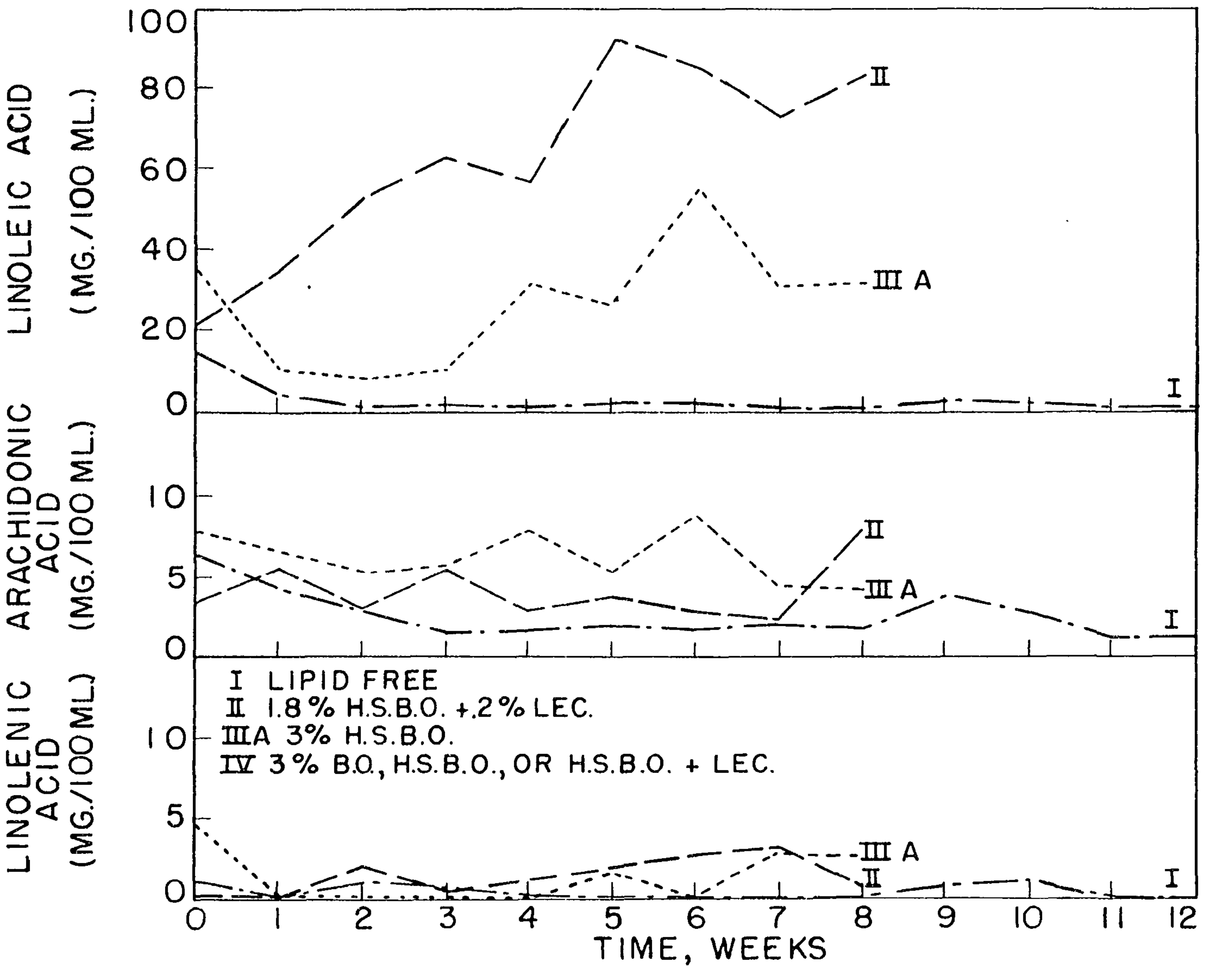

Figure 6. Effect of dietary on changes in group mean linoleic, linolenic and arachidonic fatty acids in blood plasma

Table 38 Effect of Dietary on Blood Plasma Linolenic Acid Values

Dietary Group	Sub-group	Calf No.	Breed	Sex	Syn. Milk[1]	Ration Lipid	Age in Days 0	7	14	21	28	35	42	49	56	63
							(mg./100 ml.)									
I		3596	H	F	16	None	2.1	0.0	0.0	0.0	0.6	0.5	0.2	0.0	1.0	
					11	2% H.S.B.O.										1.4
					11	2% B.O.										
		3597	H	F	16	None	2.9	0.1	4.8	2.0	0.3	1.7	0.0	0.0	0.0	0.0
					11	2% B.O.										
					11	2% B.O. + 2% Lec.										
		3603	H	M	16	None	0.0	0.8	0.3	1.7	0.2	0.0	0.4	0.0	0.0	
					13	1.5% C.S.B.O.										5.5
					11	1.5% C.S.B.O. + 1.5% Lec.										
		3768	MS	M	16	None	0.0	-	-	-	-	0.0	0.0	0.0	0.0	
					16	7.5 g. Lec.										0.2
					13	2.8% H.S.B.O. + .2% Lec.										
		3780	H	M	16	None	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.6	
					16	Pork Liver ⇔ 15 g. Fat										0.3
		3773	H	M	16	None	1.3	1.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	
					16	5 g. Methyl Esters										0.4
					16	15 g. Methyl Esters										
		3699	H	M	16	None	-	-	-	0.0	0.3	0.4	0.0	1.4	0.3	0.0
					11	2% C.S.B.O.										
					11	1.8% C.S.B.O. + .2% Lec.										
		3700	H	M	16	None	-	-	-	0.6	0.0	0.2	0.2	0.0	0.0	2.9
					11	2% H.S.B.O.										
					11	1.8% H.S.B.O. + .2% Lec.										
Average 0-8 Weeks on Fat-free Ration							1.0	0.4	1.0	0.6	0.2	0.4	0.1	0.2	0.2	
Average 8-12 Weeks on Fat-free Ration																1.0
II		3657	BS	M	11	1.8% H.S.B.O. + .2% Lec.	0.8	0.0	0.7	0.0	1.1	4.0	3.1	2.1	0.5	
		3643	BS	M	11	1.8% H.S.B.O. + .2% Lec.	0.0	0.0	3.6	1.2	1.2	0.7	2.2	4.2	1.1	
Average							0.4	0.0	2.2	0.6	1.1	2.3	2.6	3.2	0.8	
III	A	3686	H	M	11	3% H.S.B.O.	4.8	0.6	0.0	0.0	0.0	1.8	0.0	2.8	2.0	
V	A	3640	BS	M	16	5 g. Lec.	0.1	0.0	0.4	1.6	1.3	1.1				
	B	3786	BS	M	16	5 g. F.F.A.	0.0	0.0	0.0	0.0	0.1	0.0				
					16	15 g. F.F.A.							0.0			
					13	2.8% H.S.B.O. + .2% Lec.								0.0	0.0	
	C	3784	H	M	16	5 g. F.F.A.	0.0	0.0	0.0	0.0	0.0	1.3				
					16	15 g. F.F.A.							0.0	1.8	0.0	
					11	1.8% H.S.B.O. + .2% Lec.										0.9

[1] Per cent of body weight daily.

Table 38 Effect of Dietary on Blood Plasma Linolenic Acid Values

	Age in Days																				
	0	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140
					(mg./100 ml.)																
	2.1	0.0	0.0	0.0	0.6	0.5	0.2	0.0	1.0												
O.										1.4	0.0	1.9									
													2.5	4.3							
	2.9	0.1	4.8	2.0	0.3	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0								
														2.9	3.0						
2% Lec.																5.7					
	0.0	0.8	0.3	1.7	0.2	0.0	0.4	0.0	0.0												
B.O.										5.5	2.2	5.7	6.9	11.3							
B.O. + 1.5% Lec.															7.9	9.6					
	0.0	-	-	-	-	0.0	0.0	0.0	0.0												
c.										0.2	0.0	0.3	0.8	1.2	0.0	0.6					
B.O. + .2% Lec.																					
	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.6												
r ⇔ 15 g. Fat										0.3	0.0	0.0	0.0								
	1.3	1.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0												
yl Esters										0.4	0.0	0.1	0.0								
hyl Esters														0.0	0.0						
	-	-	-	0.0	0.3	0.4	0.0	1.4	0.3	0.0	0.0	0.0	0.2								
O.														2.4	4.1	3.0	5.8	4.7			
B.O. + .2% Lec.																			0.0		
	-	-	-	0.6	0.0	0.2	0.2	0.0	0.0	2.9	3.1	0.1	0.2								
O.														0.7	0.3	0.3	0.0	0.0			
B.O. + .2% Lec.																			0.8	-	-
	1.0	0.4	1.0	0.6	0.2	0.4	0.1	0.2	0.2												
										1.0	1.0	0.0	0.1								
B.O. + .2% Lec.	0.8	0.0	0.7	0.0	1.1	4.0	3.1	2.1	0.5												
B.O. + .2% Lec.	0.0	0.0	3.6	1.2	1.2	0.7	2.2	4.2	1.1												
	0.4	0.0	2.2	0.6	1.1	2.3	2.6	3.2	0.8												
O.	4.8	0.6	0.0	0.0	0.0	1.8	0.0	2.8	2.0												
	0.1	0.0	0.4	1.6	1.3	1.1															
A.	0.0	0.0	0.0	0.0	0.1	0.0															
.A.							0.0														
B.O. + .2% Lec.								0.0	0.0												
A.	0.0	0.0	0.0	0.0	0.0	1.3															
.A.							0.0	1.8	0.0												
B.O. + .2% Lec.										0.9	0.0	0.0	0.0	0.0							

V. DISCUSSION

The results of the data presented in this manuscript indicate that dietary lipids are required both quantitatively and qualitatively by the young calf for normal growth and development. Calves failed to grow normally after only a few weeks on the lipid-free diet and other deficiency syndromes began to appear at approximately 7 weeks of age. Animals on an iso-caloric diet containing 1.8 per cent hydrogenated soybean oil and 0.2 per cent lecithin made substantial weight gains and appeared to be healthy in all instances, indicating adequacy of the ration. These results with calves are in agreement with findings regarding the lipid requirements of the rat (25, 43, 116, 124), pig (138), mouse (19, 130), chicken (108) and dog (67).

The semi-synthetic milk used in producing the fat-deficiency syndrome was extremely low in fat. Every precaution was taken to remove all possible lipids from ingredients used in compounding the milk. Vitamin test casein (washed in hot alcohol) and C. P. lactose were the two most probable sources of small amounts of material. For this reason, these two ingredients were subjected to careful chemical analysis to determine the amounts of the essential

Table 39

Total Fatty Acids and Polyunsaturated Fatty Acids in Vitamin Test Casein and C. P. Lactose

Ingredient	Total Free Fatty Acids	Polyunsaturated Fatty Acids: Linoleic	Linolenic	Arachidonic
	(mg./100 g.)	(mg./100 g. total fatty acids)		
Vitamin test casein	2.38	24.97	2.69	0.00
C. P. lactose	1.24	15.20	1.39	3.68

fatty acids present. The results of these analyses are presented in Table 39.

Further calculations indicate that a 100 pound calf receiving milk at the rate of 16 per cent of its body weight each day would be consuming only about six mg. of fatty acids daily from the diet. This amount seems to be almost negligible since 15 to 20 mg. of linoleate have been found to be the apparent minimum daily requirement for rat response (24, 35, 90, 103).

In an attempt to determine the quantitative and qualitative dietary lipid requirements for the calf, five grams of methyl esters (approximately 50 per cent methyl linoleate) were homogenized into the ration of calf 3773 each day after 56 days of age. This calf seemed to respond slowly to the lipid therapy (equivalent to 2.5 grams of methyl linoleate per day) by manifesting increased daily weight gains. When the lipid was increased to 15 grams per day (7.5 grams of methyl linoleate per day) there was a marked improvement in daily weight gain and general appearance. These results suggest that the dietary lipid requirement for this calf was between 2.5 and 7.5 grams of methyl linoleate.

Calf 3780 responded positively to 15 grams of pork liver fat added to the ration at eight weeks of age. From the analysis of the pork liver fat it was calculated that this calf received approximately 2.6 grams of linoleic acid and 2.8 grams of arachidonic acid each day. Since Greenberg et al. (57) found methyl arachidonate to have 3.5 times the biopotency of the linoleic acid, this would suggest that the calf received essential fatty acids equivalent to 12.6 grams of linoleic acid per day.

Other calves (3784 and 3786), which received five grams of free fatty acids (approximately 50 per cent linoleic acid) homogenized in their milk twice daily from birth to five weeks responded poorly. Both calves had severe diarrhea, reacting similarly to calves fed relatively large amounts of unsaturated fats (10, 59, 60, 61, 78).

During the subsequent three weeks, calf 3784 was fed 15 grams of free fatty acids (approximately 7.5 grams of linoleic acid) daily. Incidence of diarrhea was reduced and daily weight gains and general appearance were somewhat better as the calf became adjusted to the ration indicating that the essential fatty acid requirments were being met.

Calf 3786 lost weight almost constantly while on this dietary regimen. At the beginning of the sixth week, the free fatty acids were increased to 15 grams per day (7.5 grams of linoleic acid) but this change seemed only to intensify the excessive diarrhea and it was necessary to change the dietary lipid at the end of this week in order to save the life of the calf.

It is possible but not probable that calf 3786 did not receive sufficient unsaturated fatty acids for normal growth and development. It seems more likely that the high incidence of diarrhea was caused by irritation of the gastrointestinal mucosa by the free fatty acids, thus causing a high incidence of diarrhea which in turn effected normal growth and development of this calf. The calf made an astounding recovery when the free fatty acids were removed from and hydrogenated soybean oil and lecithin were added to the synthetic milk. This rapid recovery suggests that some linoleic acid probably was stored in adipose tissue, aiding in the rapid recovery when the dietary lipid was changed.

Samples of lipids used in the experiment were analyzed for polyunsaturated fatty acids (21, 100) to enable

Table 40

Polyunsaturated Fatty Acid Content of
Various Lipids

Dietary Lipid	Linoleic	Linolenic	Arachidonic
		(per cent)	
Butter oil	2.87	0.57	0.44
Hydrogenated soybean oil	0.39	0.07	0.00
Crude soybean oil	58.27	11.03	0.00
Lecithin	25.53	2.69	0.00

calculation of the amounts of essential fatty acids in the various diets. The results are shown in Table 40.

Calf 3640 received daily from the beginning of the experimental period, five grams of lecithin homogenized into the milk. The calf seemed to react in the same way as calves receiving large amounts of highly unsaturated fats in their diets (10, 60, 78, 97) i.e., loss of weight and excessive diarrhea. The data presented in Table 40 show that the calf received each day 1.28, 0.13 and 0.00 grams of linoleic, linolenic and arachidonic acids, respectively. This would not seem to be adequate for the needs of the calf. Because of the critical condition of the calf it was removed from the experiment at five weeks of age in an attempt to save its life.

Calf 3768 received lecithin at a slightly higher level (7.5 grams per day) at eight weeks of age, in an attempt to effect recovery from fat deficiency. The calf lost weight during the subsequent four weeks on lecithin therapy (1.91 grams linoleic and 0.20 grams of linolenic acid per day). Recovery was satisfactory during the following three weeks when the lecithin intake was increased to 0.2 per cent and hydrogenated soybean oil (3.64 grams of linoleic and 0.44 grams of linolenic per 100 pounds of body weight per day) also was added to the ration. Thus, the increased intake of polyunsaturated fatty acids may have been responsible for the improved growth. However, Greenberg *et al*. (56) found that the growth rate increased in fat-depleted rats when fat was administered in addition to the essential fatty acids. It is entirely possible therefore that in the present investigation part of the beneficial effect of hydrogenated soybean oil therapy to calves is due to fat *per se*.

An unsatisfactory response was obtained when crude soybean oil (58 per cent linoleic and 11 per cent linolenic acid) was fed alone or with lecithin. The diarrhea produced by that lipid combination appeared to have confounded the beneficial results which might have been derived from the essential fatty acids (10, 60, 78, 97).

Butter oil was fed to 3688 throughout the 12 week feeding trial and to 3596 and 3597 after the lipid depletion period in an attempt to obtain recovery. Calf 3688 received three per cent fat in the milk which was fed at the rate of 13 per cent of body weight per day. This provided daily approximately 5.1, 1.0 and 0.8 grams linoleic, linolenic and arachidonic acids, respectively, per 100 pounds of body weight. This calf, during the 12-week feeding period, made more rapid and more efficient gains than any of the other calves. The high content of polyunsaturated fatty acids may have been responsible for the excellent response of this calf. Calf 3597 was on the fat-depletion diet for 12 weeks and continued to lose weight for a short time after the diet was supplemented by butter oil. Before termination of the experiment, however, this calf responded to the butter oil as evidenced by weight gains and a return to more normal appearance. Calf 3596 on the fat-depletion diet for only eight weeks responded readily to butter oil therapy. The difference between the response of 3597 and 3596 may have been associated with the extent of lipid depletion. Calf 3597 was on the lipid-free ration for four weeks longer than 3596 and was in a critical condition when lipids were added to the ration.

Hydrogenated soybean oil was fed as the only lipid to some of the calves while others received hydrogenated soybean oil and lecithin. Early in the experiment, it was observed that the calves receiving hydrogenated soybean oil plus lecithin consistently made larger weight gains than those receiving hydrogenated soybean oil as the only lipid supplement. This is illustrated in Figure 7. A summary of the

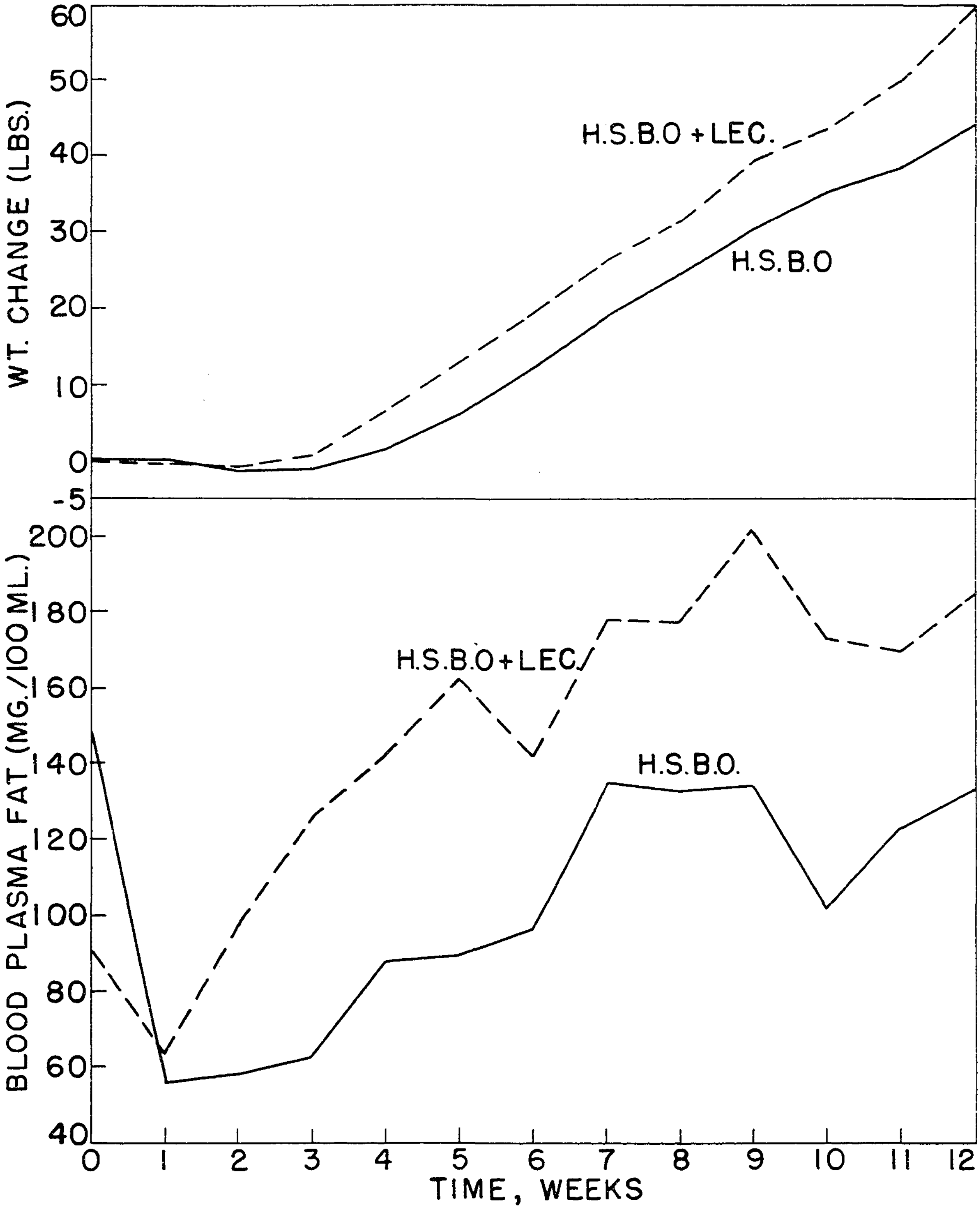

Figure 7. Effect of phospholipid on group mean weight change and blood plasma "Allen fat" values

weight gains and TDN per 100 pounds of body weight for the 0 to 8 and 8 to 12 week periods are presented in Table 41. During the first feeding period the calves which received hydrogenated soybean oil plus lecithin were on a slightly lower caloric intake than calves which received only hydrogenated soybean oil yet the former group made the larger and more efficient weight gains. During the 8 to 12 week feeding period the caloric intakes of the two groups of calves were identical but the group fed hydrogenated soybean oil plus lecithin continued to make faster and more efficient weight gains.

Calves which received milk containing three per cent hydrogenated soybean oil, fed at the rate of 13 per cent of body weight daily, consumed only 0.69, 0.12 and 0.00 grams of linoleic, linolenic and arachidonic acids, respectively, per 100 pounds of body weight. Calves fed 2.8 per cent hydrogenated soybean oil and 0.2 per cent lecithin received 3.64, 0.44 and 0.00 grams of linoleic, linolenic and arachidonic acids, respectively. These data indicate that the calves fed hydrogenated soybean oil as their only lipid source received an insufficient amount of unsaturated acids for maximum growth and feed efficiency. However, the growth of these animals exceeded that of calves on the non-fat diet supplemented with 2.5 grams of linoleic acid per day.

Table 41 Effect of Addition of Lecithin to a Semi-synthetic Milk-Hydrogenated Soybean Oil Diet

Group	Calf No.	0-8 Weeks: Wt. Gain from 0-8 Weeks (lbs)	0-8 Weeks: Av. TDN per 100 lbs. Body Wt.[1] (lbs)	0-8 Weeks: TDN per lb. Gain (lbs)	8-12 Weeks: Wt. Gain from 8-12 Weeks (lbs)	8-12 Weeks: Av. TDN per 100 lbs. Body Wt.[1] (lbs)	8-12 Weeks: TDN per lb. Gain (lbs)	8-12 Weeks: Total Wt. Gain 12 Weeks (lbs)
H.S.B.O. + Lecithin	3657	37.0	1.2	2.66				
	3643	22.0	1.3	3.62				
	3671	32.0	1.2	2.25	30.5	1.1	1.7	62.5
	3701	32.5	1.5	2.74	25.0	1.3	2.5	57.5
	3697	33.5	1.8	2.94	28.5	1.6	2.5	62.0
Average		31.4	1.4	2.84	28.0	1.4	2.2	59.4
H.S.B.O.	3686	15.5	1.3	5.60	23.0	1.3	2.7	38.5
	3698	31.5	1.3	3.45	14.0	1.0	5.0	45.5
	3696	27.5	1.8	3.63	22.0	1.7	3.0	49.5
Average		24.8	1.5	4.22	19.7	1.4	3.5	44.5

[1] Based on average weight of calves.

From data previously presented it appears that between 2.5 and 7.5 grams of linoleic acid are required per calf daily for normal growth of the calf when no fat is fed and between 0.8 and 4.1 grams per 100 pounds of body weight when fat is included in the ration. Based on a limited number of observations, this would suggest that fat contains some unidentified factors and/or fat *per se* has a sparing effect upon polyunsaturated fatty acid requirements of the calf.

This appears to be contrary to the observations of Deuel *et al*. (34) who found that hydrogenated coconut oil had a depressing effect on the growth of rats fed a low-fat diet, however, this fat contained no unsaturated fatty acids. The depressing effect was completely counteracted by adequate supplementation with linoleate. Kummerow *et al*. (87) observed that all young from rats receiving five per cent of a commercial hydrogenated fat as their only lipid source died within 72 hours after birth. Other rats treated similarly but receiving methyl linoleate supplementation reared 80 per cent of their young, thus showing the inadequate quantity of the essential fatty acids in hydrogenated fat for the rat.

There are indications that lecithin aids in fat absorption. Jones *et al*. (83) demonstrated increased absorption of fat and fat-soluble substances by the addition of an emulsifying agent such as lecithin. Kastelic *et al*. (84)

concluded on the basis of limited data that to avoid digestive disturbances in the young calf it was necessary to homogenize cottonseed oil in the presence of soy lecithin which acted as an emulsifying agent. Figure 7 further indicates that the blood plasma fat values were higher in calves receiving hydrogenated soybean oil plus lecithin than in those receiving only hydrogenated soybean oil. This suggests that hydrogenated soybean oil may be more completely absorbed when lecithin is added to the ration. However, Chung and Shaw (29) found that intravenous injections of a surface-active agent resulted in a marked increase in all plasma lipids except phospholipid; thus, they may in some manner affect fat metabolism, and therefore alter plasma fat levels, without changing the digestibility of the fat.

In this study the plasma lipid values were found to be very low at birth. These findings are in agreement with those of Allen _et al_. (3) who studied a number of blood samples taken from calves before their first nursing and found the plasma fat to be undetectable by the "Allen fat" procedure. Moreover, it has been shown (4, 5) that the polyunsaturated fatty acids in calf plasma are extremely low at birth and that the levels increase subsequent to colostrum ingestion. Zaletel _et al_. (140) studied the plasma lipids of calves from birth to four days of age and found that all values increased markedly from very low initial levels. Because of the small store of essential fatty acids by the calf at birth and the limited colostrum intake in the present study, fat deficiency syndromes probably developed earlier than might have occurred if the semi-synthetic milk had been introduced at a later age.

Two male calves, 3699 and 3700, which were not muzzled, consumed some of the wood shavings used for bedding and rumination was initiated at about seven weeks of age. Distension of the paunch was observed and this may have influenced the weight gain of these calves. From 49 days of age until they were removed from the lipid-free milk these calves had a better appearance than and their weight gains were superior to other calves in Group I. Perhaps through microorganism activity some of the essential fatty acids may have been synthesized by fermentation of cellulose in the rumen. Barki (11) reported that rats maintained on a fat-deficient diet develop fat deficiency syndromes and then begin to synthesize linoleic and linolenic acid and the deficiency symptoms disappear. While this conclusion has not been confirmed by other investigators, it is possible that microflora can multiply and, in the case of the calf, synthesize at least part of the essential fatty acids.

Although this study has indicated the essentiality of lipids in the diet of the young calf, there is need for further investigation concerning the role of unsaturated fatty acids, lecithin and of fat *per se*. Additional work needs to be done to determine the exact amount of unsaturated fatty acids required by the calf for normal growth and development. Further studies are needed to determine the contribution of lecithin in restoring growth and development to the fat-deficient calf. There is still some question as to whether the beneficial effect of lecithin is due to its contribution of unsaturated fatty acids or to some other component or function of the lecithin molecule. The intravenous injection of lipids should be attempted in these studies since this technique offers attractive possibilities. Radioactive tracers also could be employed advantageously to study lipid metabolism in the calf. Work also needs to be done to study further the relationship of dietary lipids to incidence of diarrhea.

VI. SUMMARY

The dietary essentiality of lipids for calves was studied by feeding a lipid-free, semi-synthetic milk containing vitamin-free casein, C. P. lactose, minerals and vitamins. Five of the calves on the lipid-free ration received lipid supplementation subsequent to eight weeks of age and the remaining three calves received lipid supplementation after the twelfth week. In most instances, as lipids were added to the ration, milk intake was reduced to maintain a constant caloric intake.

Twelve other calves received a similar diet plus various lipids. Weight records were kept and clinical observations were made daily on each calf. Blood plasma samples were analyzed for total fatty acids, "Allen fat", phospholipids and for linoleic, linolenic and arachidonic acids.

Fat deficiency syndromes first appeared in the calves after six weeks on the lipid-free diet and were quite severe in 50 per cent of the calves after eight weeks. The fat deficiency syndromes included scaly dandruff and loss of hair on the back, shoulders and tail. The hair remaining on the body was long, dry and lacked the luster of the hair of the calves receiving lipids. Alopecia occasionally was noted

on the neck and tail of calves on the lipid-free diet. Diarrhea became more common as the calves approached 56 days on the lipid-free milk.

Gain in body weight of the lipid-free calves was greatly retarded. The mean weight gain from 0 to 56 days was 10.9 pounds for calves fed the lipid-free diet as compared to 30.4 pounds for those which received an iso-caloric diet containing 1.8 per cent hydrogenated soybean oil and 0.2 per cent lecithin.

Response to fat supplementation by calves previously receiving lipid-free milk was dependent in part upon the extent of fat depletion before supplementation was begun. Butter oil alone and hydrogenated soybean oil plus lecithin were found to be most effective in promoting recovery from the fat deficiency syndrome.

Blood plasma "Allen fat" levels were significantly lower in the calves receiving the lipid-free milk than in the calves receiving lipids but at no time did the mean blood fat values drop below 15 mg. per cent in the lipid-free group. Calves receiving hydrogenated soybean oil as the only lipid supplement had significantly lower plasma "Allen fat" values than did calves receiving hydrogenated soybean oil and lecithin.

Blood plasma phospholipids were consistently lower in the calves on the lipid-free diet. Phospholipid values were higher in calves receiving hydrogenated soybean oil plus lecithin than in calves receiving only hydrogenated soybean oil and both were higher than those of calves fed the lipid-free diet. Total plasma fatty acid values were significantly higher in the lipid-fed calves than in the calves on lipid-free diets. Blood plasma linoleic acid was significantly higher in the calves receiving lipids than in calves on the lipid-free diets. There was little difference in the blood plasma linolenic acid content of the various dietary groups. In some of the plasma samples no linolenic acid could be found. The arachidonic acid values were slightly higher in the calves receiving lipids but were comparatively low in all animals.

VII. CONCLUSIONS

The data seem to support the following conclusions:

(1) The calf requires unsaturated fatty acids for normal growth and development. It appears that fat *per se* also acts as a growth stimulus when unsaturated fatty acids are present in the ration.

(2) The fat deficiency symptoms were retardation of growth, scaly dandruff and loss of hair over back, shoulders and tail. The hair remaining on the body was long, dry and lacked the luster of that of calves receiving lipids. Calves which received no lipids occasionally had alopecia on the neck and tail and incidence of diarrhea was high.

(3) Calves which received hydrogenated soybean oil plus lecithin grew at a faster rate than calves fed hydrogenated soybean oil as the sole lipid. The benefits derived from adding lecithin probably were due to the unsaturated fatty acids supplied by the lecithin.

(4) Plasma "Allen fat" levels of calves which received hydrogenated soybean oil plus lecithin are higher than those of calves fed hydrogenated soybean oil as the only lipid.

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